SYNTHESIS AND CHARACTERIZATION OF QUINONE METHIDE PRECURSORS AS ACETYLCHOLINESTERASE REACTIVATORS

Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation with research distinction in Chemistry in the undergraduate colleges of The Ohio State University

by

Sarah M. Ercegovic

The Ohio State University

June 2011

Project Advisor: Dr. Christopher S. Callam, Department of Chemistry

ABSTRACT

Acetylcholinesterase (AChE) is an essential enzyme in the human body, which degrades acetylcholine into choline for reuse in the nervous system near neuromuscular junctions. The use of chemical war agents, such as tabun, inhibit and age AchE, causing significant nerve damage. Previous studies have shown that high energy quinone methides (QM) could potentially reverse the damage done to the active site on aged AChE through a kinetically favored alkylation of a phosphodiester. This research focuses on the synthesis of quinone methide precursors and their nucleophilic reactivity, which is comparable to the proposed enzyme reactivation reaction. Synthetic methods have centered on a reductive amination pathway to produce salts derived from vanillin and 3-chloro-4-hydroxybenzaldehyde with various amines. Current results confirm the structure of amine salts produced from vanillin and 3-chloro-4-hydroxybenzaldehyde by ^{1}H NMR and ^{13}C NMR. The reactivity of these salts towards substitution has been tested with multiple nucleophiles under a variety of conditions. The reactions have proved to be successful on all of the amine salts. Mechanistic understanding on the formation of high energy quinone methides and optimization of the reactions for their formation is currently still in progress. Reaction testing with other nucleophiles will continue as well.

ACKNOWLEDGEMENTS

I would like to thank my research advisor, Dr. Christopher Callam, for giving me the opportunity to conduct research in his laboratory. Through his guidance, knowledge and motivation, I have been able to truly develop a passion for organic chemistry and the infinite directions it can take in scientific research.

I would like to thank Mr. Michael R. Woodruff for his help and guidance as I initially began this project. So many questions were posed and answers found as a result of his presence in the laboratory this past fall.

I would like to thank Dr. Christopher Hadad, Dr. Carolyn Reid and Ali Esfahani for their help in collecting some of this data in the results and discussion portion of this writing.

I would like to thank Dr. Patrick M. Woodward and Dr. Matthew W. Stoltzfus for their mentorship through the Research Experience for Enhanced Learning (REEL) Program. They initially inspired me to pursue research not only as an undergraduate, but also in my future career as a chemist.

I wish to thank my family, whose constant and unconditional guidance, encouragement, prayers and support have made my undergraduate career possible. They have sacrificed and provided more than I could ever ask of them. Finally, thanks be to God for his unending faithfulness—his plans for me have truly been good.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

1. INTRODUCTION

Quinone methides (QM) are proposed to be essential intermediates in a large number of biological processes[1-2] such as enzyme inhibition,[4] DNA-alkylation[5] and crosslinking.[5] Regarding enzyme inhibition, they have been shown to particularly inhibit serine hydrolase,[6] *β*-lactamase,[6] phosphatase,[4] and ribonuclease.[7] A vast amount of previous research has been conducted on QMs, both *o*-QM and *p*-QM and their reactivity. Figure 1.1 shows the different structures of QMs that can exist. *o*-QM and *p*-QM are the only closed shell species for which electrophilic and nucleophilic reactions can occur. The *m*-QM is instead a diradical open shell species.[8] A large amount of previous research exists with respect to the *o*-QM and its reactivity towards alkylation. Two of the reasons for the reactivity of QMs are its ability to act as a Michael acceptor as well as the driving force of the structure's return to aromaticity.

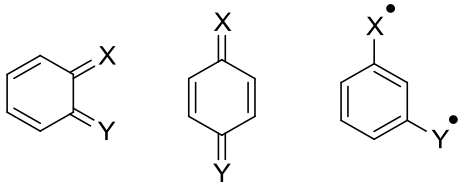


Figure 1.1. General quinone methide (QM) structure where X=O and Y=CH_2.

QMs exhibit electrophilic character[8] at the exocyclic methylene to form benzylic adducts, thus obtaining a new product with an aromatic structure. The electrophilic nature of QMs makes them an attractive intermediate for biological reactions. Previous research has shown that such types of reactions have shown good conversion using sulfur-based nucleophiles.[9] An increase in rate, approximately four orders of magnitude, was seen when using a thiol versus a sulfur anion.

In the absence of a sulfur nucleophile, nitrogen alkylation has been tested and obtained in good yields.[9] Additionally, kinetic probing studies have shown that the half-life of the *p*-QM compared to *o*-QM is approximately two orders of magnitude as large (2 ms versus 208 ms).[10,11] It is of interest to investigate if the longer half-life of the *p*-QM could yield better electrophilic reactivity and ultimately larger yields of adducts. It should be noted that these results were seen using only *o*-QM. The reactivity of these potential QMs with various nucleophiles, especially sulfur-based nucleophiles, constitutes a large portion of this research.

The documented reactivity of QMs makes them an attractive species for aid in alkylation of phosphodiesters in DNA. Research into the reactivity of *p*-QM shows them successful in alkylation reactions with a phosphodiester, followed by lactonization of the resulting trialkyl phosphate for two functionalized QMs.[12] Shown in Figure 1.2 is the inspiration for this research which improved efforts to develop a fully functional DNA phosphodiester alkylating agent. The efforts to incorporate the phosphate as an acceptor of an alkyl group is part of an inspiration to attempt to develop an efficient way to reverse the adverse effects of organophosphorus nerve toxins.

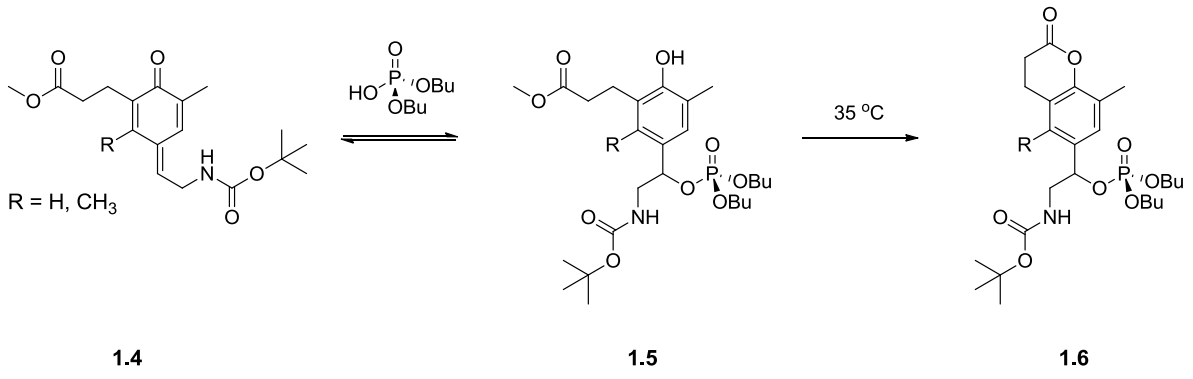


Figure 1.2. Favorable substitution and rearomatization drives the reaction of the QM with various organophosphates.[12]

Acetylcholinesterase (AChE) is an essential enzyme in the human body, which converts acetylcholine into choline in the nervous system. Hazardous organophosphorus compounds (OPs) shown in Table 1.1 contain a phosphate-like group that binds to Ser-203 in the AChE active site inhibiting the function of the enzyme as shown in Scheme 1.1. The inhibited enzyme can age in minutes to hours and the affected individual loses nerve sensation as well as other severe biological consequences. The use of QMs as phosphoester alkylating agents is attractive because if successful, it could alkylate the bound phosphate, reactivate AChE with the aide of oxime-based pharmaceutical agents, thus reversing the adverse effects of the nerve toxin. The QM must be generated *in situ* because of its short lived existence, thus focus on the synthesis of its precursors is the focus of this thesis.

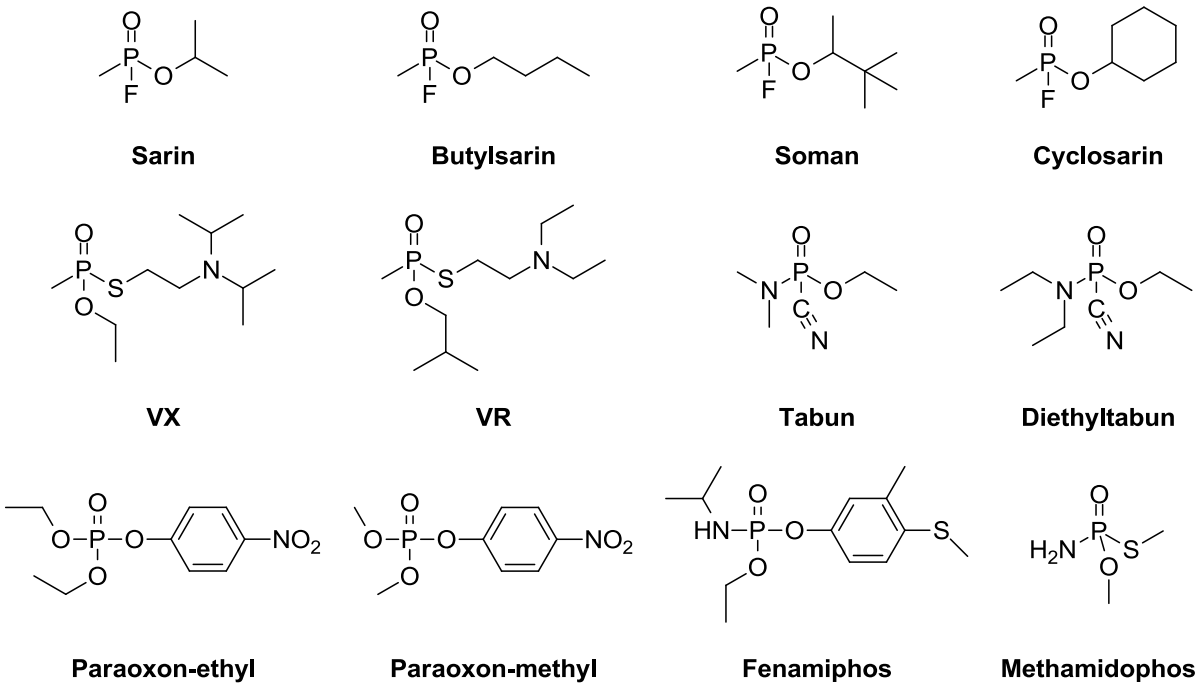
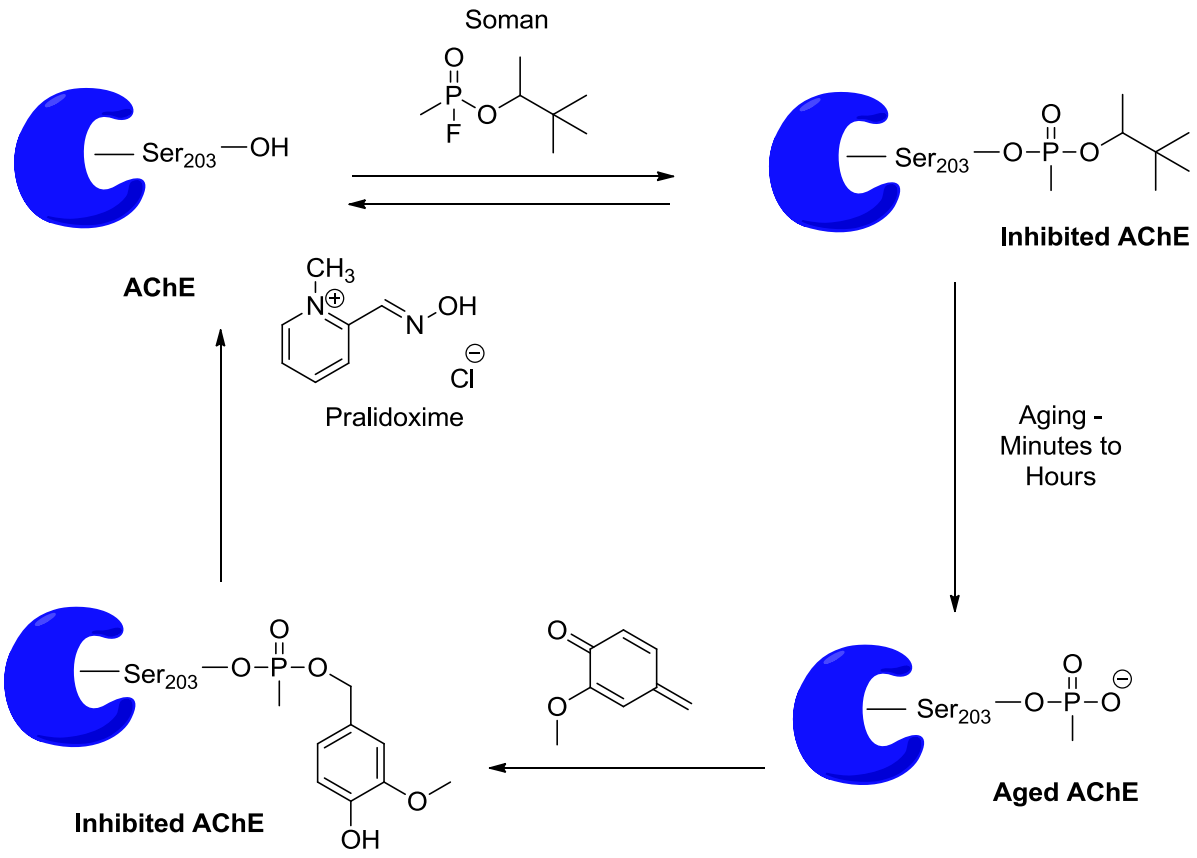
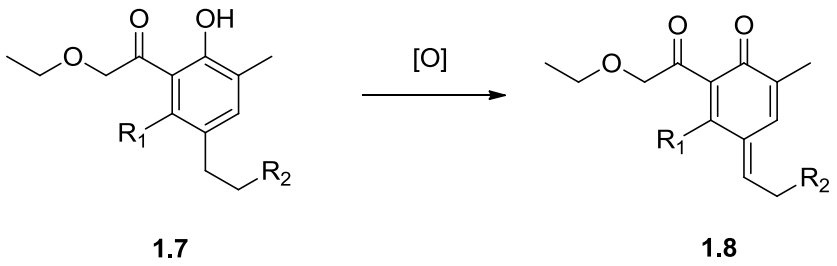


Table 1.1. Example organophosphorous nerve toxins.



Scheme 1.1. AChE inhibition and aging by OPs and reinhibition and reactivation via QMs and oximes.

There are two mechanisms for which the investigated *p*-QM precursor and *o*-QM can proceed by. Recent research shown in Figure 1.3 has shown that the oxidation of a phenol has been successful using lead(II) oxide and silver(I) oxide. The reaction specifically afforded selectively the *p*-QM and allowed the QM to be directly produced via phenolic precursors.[13-15] Phenolic derivatives functionalized at the *ortho*- and *para*- positions were shown to oxidize specifically the *p*-QM.[16]



1.7 **1.8**

Figure 1.3. Oxidation of a phenol to the QM structure using metal based oxidating agents.

Apart from the oxidative method, Weinert, et. al. investigated the rates of formation and decomposition of the QM. Apart from their previous assumptions, it was observed that the strongest nucleophiles of DNA reacted in a kinetic manner with *o*-QM. However, these kinetic products were energetically stable as the reaction was reversible. The *o*-QM was ultimately regenerated and substitution with weaker nucleophiles afforded irreversible thermodynamic products.[17,18] Their computational studies showed that electron-withdrawing and -donating groups attached directly to the QM and its precursors influenced the rate of the QM formation and the substituted products. Specifically, electron-withdrawing groups, ammonium salts, as shown in Figure 1.4, suppress the regeneration of the QM and strongly promote nucleophilic addition to these intermediates via a laser flash photolysis (LFP) procedure.[19] The stronger the electron-withdrawing group was, such as NO_2, the larger the rate constant for production of the final substituted adduct. The success in using precursors with ammonium salts can be attributed to the higher quantum yields of the salts compared to the corresponding alcohols.[10]

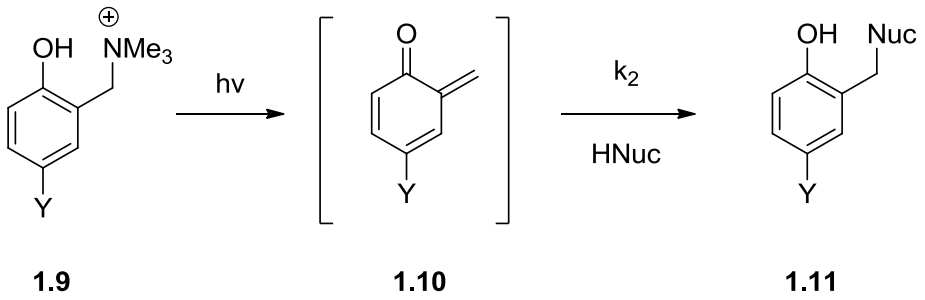


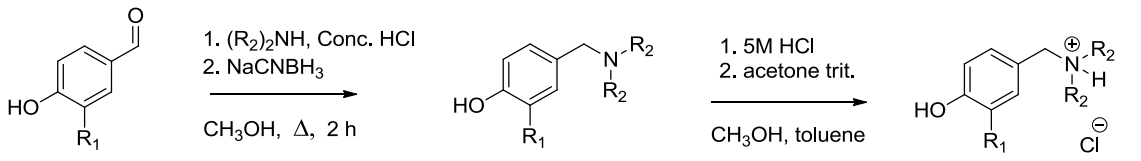
Figure 1.4. Formation and substitution of the QM.

With the documented success of using *o*-QM as a reactive intermediate, it is of interest to investigate its isomer *p*-QM as a subsequent reactive intermediate, for which studies on its reactive behavior of this sort is not published to our knowledge. Attempting to build a synthetic route and optimized methodology with the intent of producing *p*-QM precursors using economically efficient means is the main interest and goal in this research.

2. RESULTS AND DISCUSSION

2.1 Reductive Aminations. The formation of the main precursors were derived from vanillin, a commercially available, cost-effective starting material of which the synthetic sequence from starting material to ammonium salt was two steps. Through multiple synthetic steps, shown in Scheme 2.1, relatively high yields were achieved for the products shown in Table 2.1. The ease of preparation for this work can be noted by the fact that these salts were produced in a one-pot synthesis as shown in Scheme 2.1. We observed that longer reaction times produced higher yields.

The aldehyde was first mixed with the secondary amine (6 eq.) and HCl (conc.) at rt. The resulting mixture was refluxed for 2 h after the addition of $NaCNBH_3$ to produce the crude amine. The reaction progress was monitored by GC/MS to verify complete reduction of the imine. The resulting crude amine was isolated and redissolved in methanol (15 mL) and toluene (4 mL) and aqueous HCl was added to isolate the ammonium salt. Both the 3-methoxy and 3-chloro derivatives proved to give excellent yields over the two-step, 1-pot procedure.



2.1

2.1a = $R_1 = OCH_3$
2.1b = $R_1 = Cl$

2.2

2.2a = $R_1 = OCH_3$, $R_2 = CH_3$
2.2b = $R_1 = OCH_3$, $R_2 = CH_2CH_3$
2.2c = $R_1 = Cl$, $R_2 = CH_3$
2.2d = $R_1 = Cl$, $R_2 = CH_2CH_3$

2.3

2.3a = $R_1 = OCH_3$, $R_2 = CH_3$
2.3b = $R_1 = OCH_3$, $R_2 = CH_2CH_3$
2.3c = $R_1 = Cl$, $R_2 = CH_3$
2.3d = $R_1 = Cl$, $R_2 = CH_2CH_3$

Scheme 2.1. Reductive aminations using dimethylamine and diethylamine.

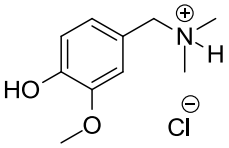
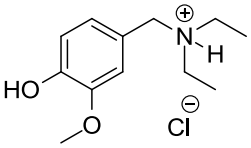
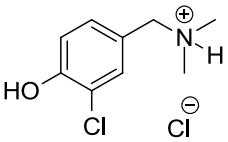
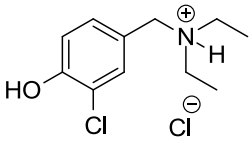
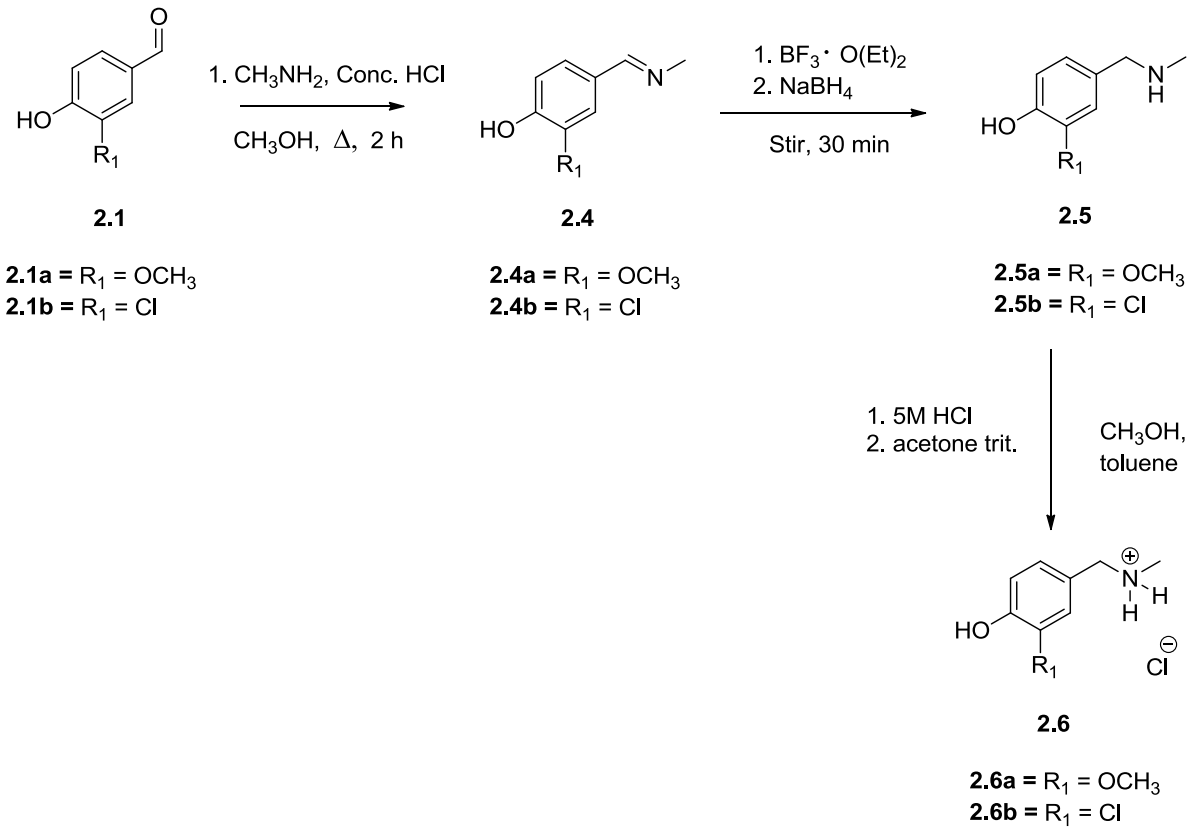
Structure				
	2.3a	**2.3b**	**2.3c**	**2.3d**
% Yield	65%	71%	76%	76%

Table 2.1. Ammonium salts and respective yields.

2.2 Reductive Aminations with Lewis Acids. The ammonium salts derived from vanillin or 3-chloro-4-hydroxybenzaldehyde and methylamine were not successful without the use of an activating Lewis acid, boron trifluoride diethyl etherate (BF_3•OEt_2). The synthetic route shown in Scheme 2.1 failed to reproduce the imine or amine products. Original attempts using titanium trichloride ($TiCl_3$) were unsuccessful due to the complications of titanium salts and assumed formations of coordination complexes. Attempts with BF_3•OEt_2 proved successful with a slightly different synthetic method shown in Scheme 2.2**.** Structures, yields and convergence of the neutral compounds via GC/MS are shown in Table 2.2. All reactions in Scheme 2.2 showed complete conversion to the corresponding imine.



Scheme 2.2. Reductive amination with Lewis acid.

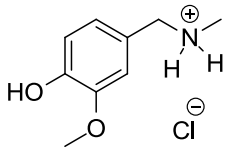
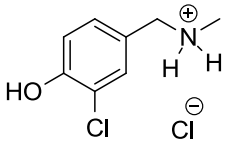
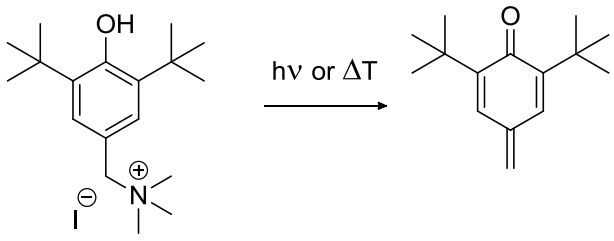
Structure		
	2.6a	**2.6b**
% Yield	65%	18%

Table 2.2. Ammonium salts from Lewis acid complexation.

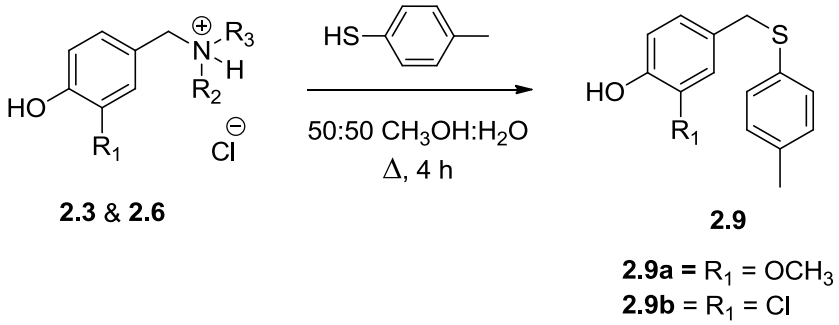
The yield for several synthetic steps for product **2.6a** was relatively high; the remaining percentage of theoretical yield can be attributed to polymerization products or decomposition of the QM precursors. The yield for product **2.6b** was considerably low; the reason for which is still under investigation. Perhaps, reasons surrounding the poor yield could be attributed to the instability of the product. This characteristic lowers the reactivity of the benzylic position towards the formation of the quinone methide. Reasons for this hypothesis can be attributed to the longer half-life (3060 s) of quinone methide **2.8** shown in Figure 2.1 due to the electron donating *tert*-butyl substituents.[20]



2.7 **2.8**

Figure 2.1. Formation of *tert*-butyl substituted *p*-QM.[20]

2.3 Nucleophilic Substitution with 4-methylbenzenethiol. Studies to evaluate the effectiveness of the ammonium salts subject to nucleophilic substitution showed positive results. Each of the ammonium salts shown in Tables 2.1 and 2.2 were subjected reactions with 4-methylbenzenethiol at 60 ^{o}C, 80 ^{o}C, and 100 ^{o}C to determine the optimal conditions for substitution. The general reaction is shown in Scheme 2.3. The convergence of products **2.9a** and **2.9b** at these temperatures are shown in Table 2.3. Results from these reactions show that a higher yield and better convergence of starting material were results of the reaction at a higher temperature.



Scheme 2.3. Nucleophilic substitution of ammonium salts with 4-methylbenzenethiol.

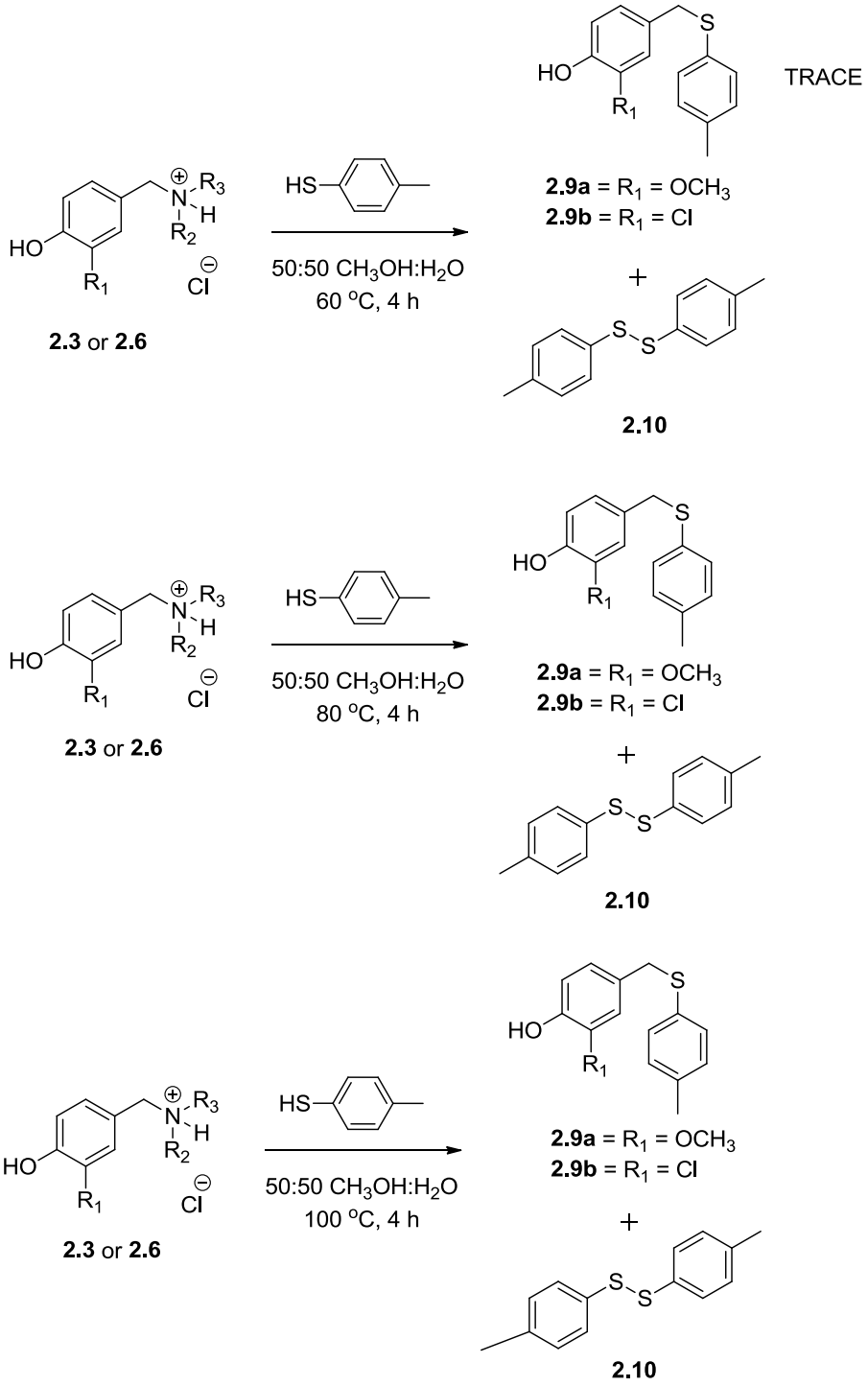
Compound	60 °C	80 °C	100 °C
2.3a	Yes; <10%	Yes	Yes
2.3b	No rxn	Yes; <10%	Yes
2.3c	No rxn	Yes; <10%	Yes
2.3d	No rxn	No rxn	Yes
2.6a	Yes; <10%	Yes	Yes
2.6b	No rxn	No rxn	Yes

Table 2.3. Temperature/time study monitoring for evidence of substitution progress.

At 60 °C, less than 10% of product **2.9a** was seen using salts **2.3a** and **2.6a**. Using salt **2.6b** afforded no traceable product **2.9b**. The major product was byproduct **2.10** (Scheme 2.4). At 80 °C, the major product was byproduct **2.10**; however, the desired product **2.9a** was identified in very small amounts. Reaction of salt **2.6b** showed no conversion to the desired product **2.9b**. At 100 °C, the substitution was observed in high yield along with **2.10**. Additionally, several separate reactions were run with **2.3b** and **2.3c** at 100 °C for 6 h showing the highest conversion to **2.9a** and **2.9b**. It can be concluded that a higher temperature over a longer amount of time results in a higher percent conversion of the substituted product.

These reactions and their products are shown in Scheme 2.4. It can be proposed that in order to optimize further the yield of the product, a longer reaction time such as 6 h, should be

exploited and thoroughly tested over all ammonium salts. Additionally, the use of a lower equivalent of 4-methylbenzenethiol should be tested. Isolated yields at 80 ^{o}C using 4-methylbenzenethiol with salts **2.3a-d** and **2.6a-b** are shown in Table 2.4. The differences in the isolated yield showed the highest conversion of compound **2.3d**.

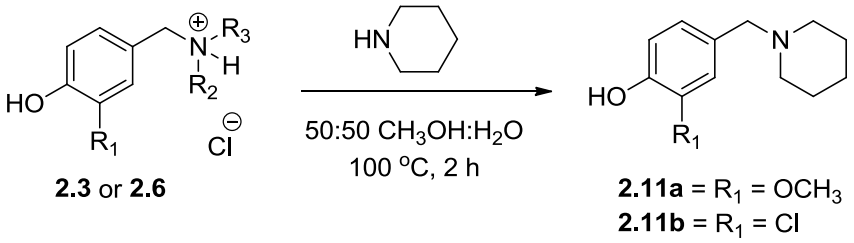


Scheme 2.4. Nucleophilic substitution temperature optimization.

Compound	R1	R2	R3	Isolated Yield (%)
2.3a	OCH_3	CH_3	CH_3	68
2.3b	Cl	H	CH_3	TBD
2.3c	Cl	CH_3	CH_3	72
2.3d	Cl	CH_2CH_3	CH_2CH_3	77
2.6a	OCH_3	H	CH_3	62
2.6b	OCH_3	CH_2CH_3	CH_2CH_3	70

Table 2.4. Isolated yields of substitution reactions with 4-methylbenzenethiol.

2.4 Experimentation with other nucleophiles. The nucleophilic reactivity of compounds **2.3a-d** and **2.6a-b** were evaluated qualitatively and qualitatively with nitrogen and oxygen based nucleophiles. The use of piperidine as a nucleophile afforded the desired product under the conditions shown in Scheme 2.5. The results of isolation of products **2.11a** and **2.11b** are shown in Table 2.5.



Scheme 2.5. Nucleophilic substitution of ammonium salts with piperidine.

Compound	R1	R2	R3	Isolated Yield (%)
2.3a	OCH_3	CH_3	CH_3	58
2.3b	OCH_3	CH_2CH_3	CH_2CH_3	70
2.3c	Cl	CH_3	CH_3	55
2.3d	Cl	CH_2CH_3	CH_2CH_3	TBD
2.6a	OCH_3	H	CH_3	TBD
2.6b	Cl	H	CH_3	TBD

Table 2.5. Nucleophilic substitution isolated yields using piperidine at 100 ^{o}C.

With the successful substitution of **2.3** and **2.6** with piperidine, qualitative testing was conducted using **2.12-2.15** shown in Table 2.6. All showed convergence to the desired substituted product. Further work is required to determine optimized conditions for isolated yields.

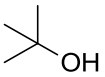
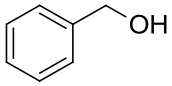
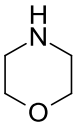
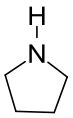
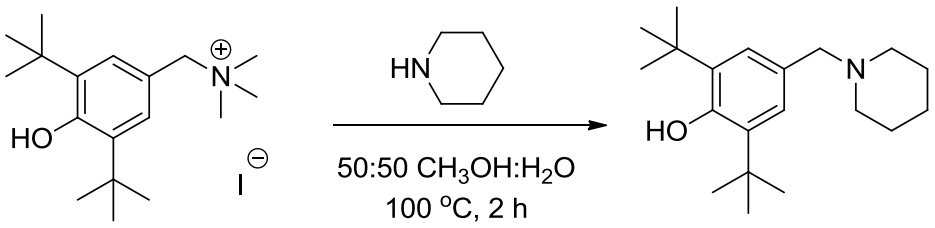
Compound	 **2.12**	 **2.13**	 **2.14**	 **2.15**
Product Conversion via GCMS?	Yes	Yes	Yes	Yes

Table 2.6. Nucleophilic substitution with nitrogen and oxygen based nucleophiles. Of the four nucleophiles above, all showed a substituted product as a result of heating to 100 ^{o}C.

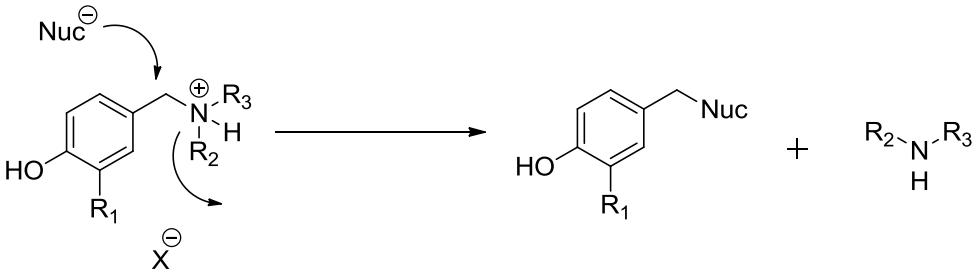
Additional substitution reactions included the use of product **2.7** with piperidine to yield **2.16** in 91% yield as shown in Scheme 2.6. It can be suggested that the electron donating characteristics of the trialkyl ammonium salts on **2.7** significantly aided the high yield of **2.16**, which as will be discussed is a case for the QM mechanism.



2.7 **2.16**

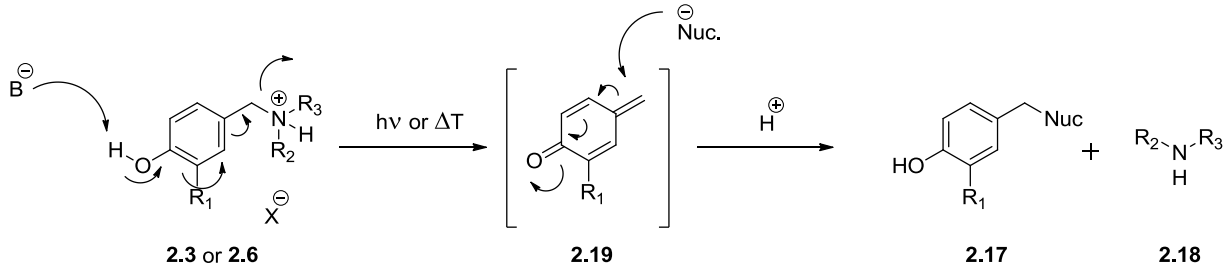
Scheme 2.6. Nucleophilic substitution of trialkyl substituted ammonium salt with piperidine.

2.5 Discussion of Potential Mechanism. The successful results of nucleophilic substitution suggest that the precursors, compounds **2.3a-d** and **2.6a-b** are indeed substituting; however, the mechanism of substitution is only speculated at this time. With substituents at the benzylic position on the aromatic ring, the system can undergo S_N2 displacement with moderate to strong nucleophiles. Scheme 2.7 illustrates this mechanism. The system could also be going through an *in situ* generated QM intermediate. Results that suggest this mechanism are the substitution results using salt **2.7** shown in Figure 2.1 with piperidine afforded a product in 91% yield. The high yield for this reaction along with the data for the half-life of QM **2.8** suggests that this reaction is proceeding by way of the QM intermediate. Scheme 2.8 illustrates this mechanism.



2.3 or **2.6** **2.17** **2.18**

Scheme 2.7. Proposed S_N2 displacement mechanism for nucleophilic substitution.



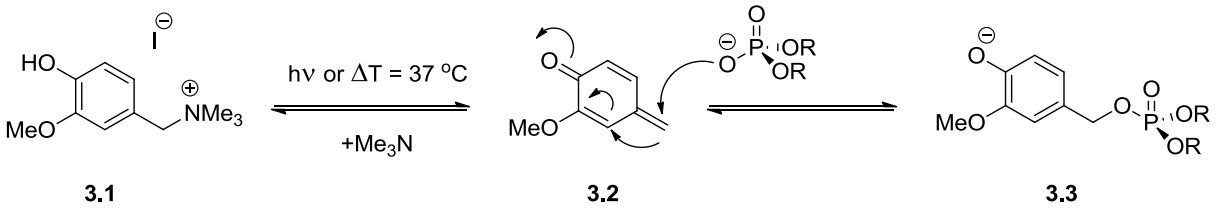
Scheme 2.8. Proposed mechanism for nucleophilic substitution by way of QM intermediate.

Both mechanisms are valid and plausible; however additional mechanistic probing studies are required for all of these reactions to ultimately determine the actual predominated pathway. Additionally, it should be noted that for the purpose for which the substitution reactions serve, which is to mimic the biological reaction during phosphodiester alkylation, the ultimate end product can be produced by either mechanism.

3. CURRENT AND FUTURE WORK

3.1 Substrate Experimentation. With the successful results using vanillin and 3-chloro-4-hydroxybenzaldehyde, current work is under way using *ortho*-vanillin. Tabulated salt yields and substitution data is yet to be determined; however, initial results show that the reductive amination methodology is successful via successful molecular weight determination on GC/MS. This substrate is of particular interest because it would afford data for a potential mechanism using an *o*-QM. As stated previously, a bulk of the original data and mechanistic studies completed on QMs was using *o*-QM.

3.2 Phosphodiester Alkylation. With the successful substitution of salts **2.3a-d** and **2.6a-b**, to produce products **2.9a-b** and **2.11a-b**, the use of a phosphdiester as the nucleophile is the next step in this research. The pathway for this next step is shown in Scheme 3.1. Should this reaction and proposed mechanism be successful, the case for using the ammonium salts as precursors to reverse the aging process of nerve gas toxins on AChE is very strong. Continued work in this area is needed to validate this hypothesis.



3.1 **3.2** **3.3**

Scheme 3.1. Proposed phosphate alkylation.

4. EXPERIMENTAL

4.1 General. Solvents were used without any type of further purification. All reactions were carried out at standard atmospheric pressure and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck) and by GC/MS on a Agilent 6850 GC with an Agilent 5975C Mass Detector on an Agilent HP-5MS column (30 m × 0.250 mm, film 0.25 μm). Spots were detected by UV light or by phosphomolybdic acid (PMA) stain followed by heating. Organic solutions of crude products were dried using anhydrous Na_2SO_4. Chromatography was performed on silica gel 60 (40-60 μm). ^{1}H NMR spectra were recorded at 250 MHz and chemical shifts are referenced to TMS (0.0, $CDCl_3$, CD_3OD). ^{13}C NMR were recorded at 62.9 MHz and ^{13}C chemical shifts are referenced to $CDCl_3$ (77.00, $CDCl_3$, CD_3OD). IR spectroscopy was performed using KBr discs. Product yields from amination reactions and nucleophilic substitution reactions are given in Tables 2.1-2.6.

4.2 General Procedures for Reductive Amination and Salt Formation

Method A. Methylamine (4.1 mL, 47 mmol, 6 eq.) was stirred with methanol (20 mL) while concentrated HCl (0.7 mL) was added dropwise over 2-3 min. The reaction was covered until HCl fumes cleared. Vanillin (1.2 g, 7.9 mmol, 1 eq.) or 3-chloro-4-hydroxybenzaldehyde (1.23 g, 7.9 mmol, 1 eq.) were added and allowed to stir until homogeneous. The reaction was heated to reflux for 2 h and then allowed to cool to rt. Boron trifluoride diethyl etherate (0.97 mL, 7.9 mmol, , 1 eq.) was then added dropwise. Sodium borohydride (0.328 g, 8.69 mmol, 1.1 eq.) was added in one portion and allowed to stir for an additional 30 min at rt. Distilled water (30 mL) was added to the reaction and allowed to stir for 5 min. The solution was extracted with

dichloromethane (3 x 40 mL) and the organic layers were collected and dried with anhydrous Na_2SO_4. The solution was concentrated in vacuo to yield a crude solid. This solid was dissolved in methanol (15 mL) and heated slightly to aid in complete dissolution. 5M HCl (1.52 mL) was added to the mixture to a pH of 2-3. The solution was then concentrated in vacuo at 60 ^oC until a solid or syrup remained. The mixture was allowed to sit overnight for crystallization to occur. The crude product was triturated with acetone (3 x 3 mL) and isolated after centrifuging to yield salt **2.6a** (1.05 g, 65 %) or salt **2.6b** (0.288 g, 18 %) as tan powders. **(2.6a)**: ^1H NMR (250 MHz, CD_3OD, δ_H) 6.94 (s, 1H), 6.81-6.70 (m, 2H), 3.95 (s, 2H), 3.77 (s, 3H), 3.19-3.17 (m, 1H), 2.55 (s, 3H); ^{13}C NMR (62.9 MHz, CD_3OD, δ_C) 147.9, 147.67, 122.70, 122.05, 115.23, 112.93, 55.14, 52.25, 31.38; IR (KBr discs) 2940, 2776, 2573, 2446, 2037, 1636, 1615, 1524 cm^{-1}.

(2.6b): ^1H NMR (250 MHz, CD_3OD, δ_H) 7.47-7.46 (m, 1H), 7.25 (dd, 1 H, J = 2.5, 7.5 Hz), 6.98 (d, 1H, J = 10 Hz), 4.08 (s, 2H), 3.32-3.29 (m, 1H), 2.68 (s, 3H); ^{13}C NMR (62.9 MHz, CD_3OD, δ_C) 155.71, 132.85, 132.60, 130.98, 124.40, 122.21, 118.08, 116.96, 52.68, 32.97; IR (KBr discs) 3551, 3479, 3414, 3237, 2982, 1638, 1617 cm^{-1}.

Method B. Dimethylamine (5.8 mL, 46 mmol, 6 eq.) or diethylamine (4.7 mL, 46 mmol, 6 eq.) was stirred with methanol (20 mL) while concentrated HCl (0.7 mL) was added dropwise over 2-3 min. After acid was added, the reaction was covered until HCl fumes cleared. Vanillin (1.2 g, 7.9 mmol, 1 eq.) or 3-chloro-4-hydroxybenzaldehyde (1.23 g, 7.9 mmol, 1 eq.) was then added to the reaction and allowed to stir until homogeneous. Sodium cyanoborohydride (1.1 eq.) was then added in one portion and the solution was stirred for at least 1 hr. The solution was heated to reflux for 2 h. After cooling to rt, distilled water (30 mL) was added and allowed to stir for 5 min. The solution was extracted with dichloromethane (3 x 40 mL) and the organic layers were collected and dried with anhydrous Na_2SO_4. The organic solution was concentration in

vacuo to yield a crude syrup. This solid was dissolved in methanol (15 mL) and heated slightly to aid in complete dissolution. 5M HCl (1.52 mL) was added to the mixture to a pH of 2-3. The solution was then concentrated in vacuo at 60^{o} C until a solid or syrup remained. The mixture was allowed to sit overnight for crystallization to occur. The crude product was triturated with acetone (3 x 3 mL) and isolated after centrifuging to yield salt **2.3a** (1.12 g, 65 %) as a white powder, **2.3b** 1.38 g, 71 %) as a white powder, **2.3c** (1.40 g, 76 %) as a white powder, or **2.3d** (1.30 g, 76 %) as a white powder. **(2.3a)**: ^{1}H NMR (250 MHz, CD_3OD, δ_H) 6.98 (s, 1H), 6.83-6.73 (m, 2H), 4.09 (s, 2H), 3.78 (s, 3H), 3.20-3.18 (m, 1H), 2.70 (s, 1H); ^{13}C NMR (62.9 MHz, CD_3OD, δ_C) 148.10, 123.80, 120.55, 115.26, 113.74, 60.93, 55.18, 41.22; IR (KBr discs) 3544, 3411, 3237, 2709, 1638, 1616 cm^{-1}.

(2.3b): ^{1}H NMR (250 MHz, CD_3OD, δ_H) 7.11 (s, 1H), 6.95-6.84 (m, 2H), 4.21 (s, 2H), 3.88 (s, 3H), 3.30-3.28 (m, 1H), 3.22-3.12 (m, 4H), 1.32 (t, 6H, *J* = 7.5 Hz); ^{13}C NMR (62.9 MHz, CD_3OD, δ_C) 149.40, 149.22, 125.20, 121.74, 116.56, 115.32, 57.34, 56.65, 9.02. IR (KBr discs) 3550, 3479, 3412, 3237, 1639, 1617 cm^{-1}.

(2.3c): ^{1}H NMR (250 MHz, CD_3OD, δ_H) 7.48-7.47 (d, 1H, *J* = 2.5 Hz), 7.26 (dd, *J* = 2.5, 10 Hz), 4.18 (s, 2H), 3.28-2.36 (m, 1H), 2.79 (s, 6H); ^{13}C NMR (62.9 MHz, CD_3OD, δ_C) 132.31, 130.49, 121.38, 120.98, 116.68, 59.81, 41.27; IR (KBr discs) 3550, 3479, 3412, 3236, 3115, 2956, 2676, 1638, 1617 cm^{-1}.

(2.3d): ^{1}H NMR (250 MHz, CD_3OD, δ_H) 7.47 (d, 1H, *J* = 2.5 Hz), 7.24 (dd, 1H, *J* = 2.5, 7.5 Hz), 6.97 (d, 1H, *J* = 7.5 Hz), 4.19 (s, 2H), 3.28-3.26 (m, 3H), 3.14 (q, 4H, *J* = 7.5 Hz), 1.29 (t, 6H, *J* = 7.5 Hz); ^{13}C NMR (62.9 MHz, CD_3OD, δ_C) 155.93, 133.67, 131.89, 122.66, 122.27, 118.03, 56.30, 47.62, 43.46, 11.52, 9.06; IR (KBr discs) 3550, 3479, 3412, 3237, 1638, 1617 cm^{-1}.

4.3 General Procedures for Nucleophilic Substitution

Reaction of Salts 2.3a-d and 2.6a-b with 4-methylbenzenethiol. 4-methylbenzenethiol (1.2 eq) was added to salts **2.3a-d** and **2.6a-b** (100 mg, 1. eq.) in 50:50 H_2O:CH_3OH (~5 mL). The reaction was allowed to heat for 4 h or 6 h at either 60 ^oC, 80 ^oC, or 100 ^oC. The reaction was allowed to cool to rt and was then extracted with diethyl ether (3 x ~3 mL). The organic layers were collected and dried with anhydrous Na_2SO_4. The dried organic layers were concentrated in vacuo to yield a crude product **2.9a** or **2.9b** as a white, clear or pale yellow residue. The crude product was purified by chromatography (15:1, petroleum ether/EtOAc) to obtain **2.9a** (0.0274 g, 25.87% as a clear residue: R_f 0.20 (15:1 hexanes/EtOAc) or **2.9b** (0.042 g, 35.12%) as a clear residue: R_f 0.20 (15:1 hexanes/EtOAc); **(2.9a)**: ^{1}H NMR (250 MHz, $CDCl_3$, δ_H) 7.20 (d, 2H, J = 7.5 Hz), 7.07 (d, 2H, J = 7.5 Hz), 6.80-6.70 (m, 3H), 3.99 (s, 2H), 3.81 (s, 3H); IR (KBr dics) 3020, 2400, 1515, 1215 cm^{-1}.

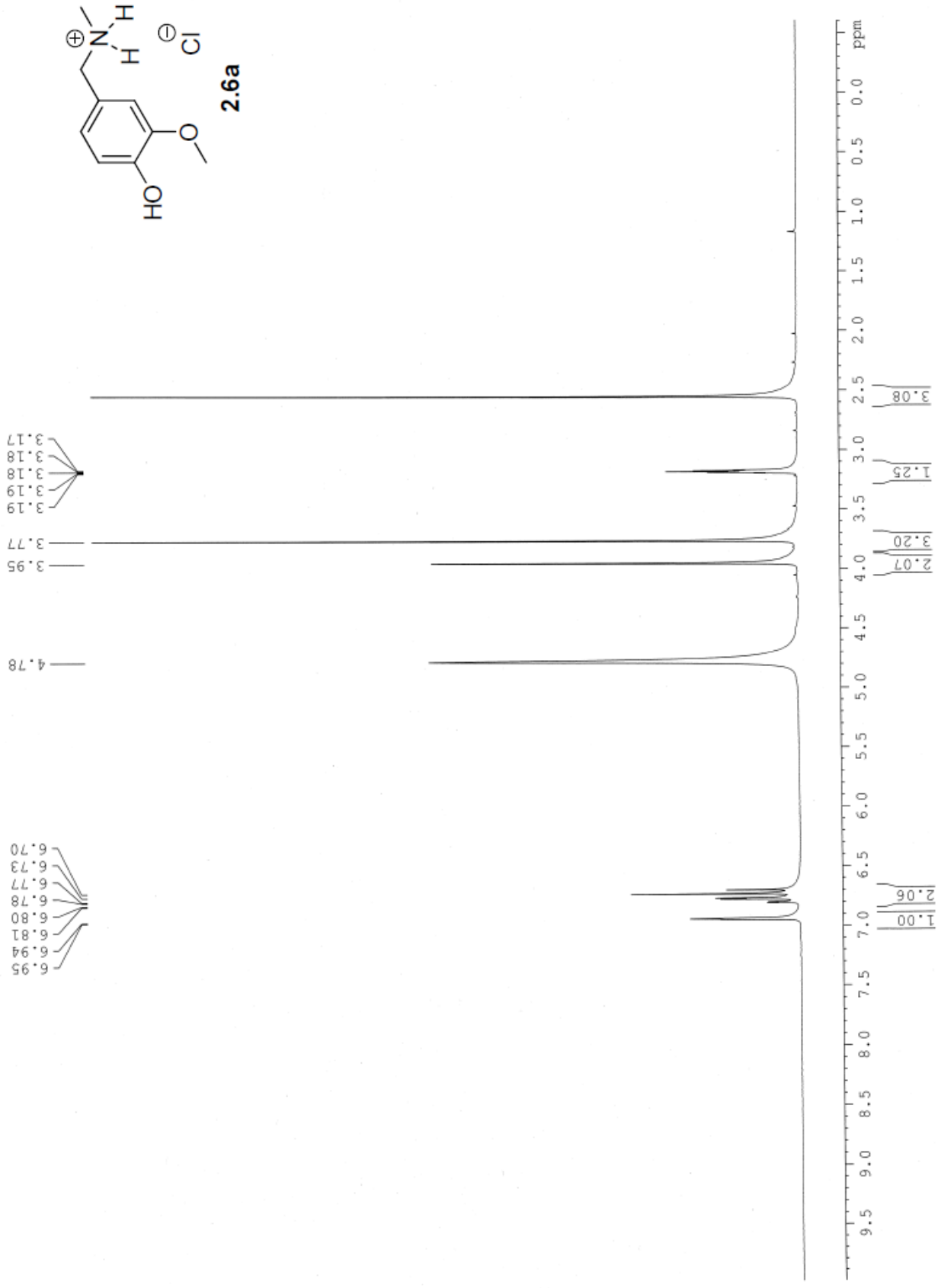
(2.9b): ^{1}H NMR (250 MHz, $CDCl_3$, δ_H) 7.18 (d, 3H, J = 7.5 Hz), 7.07-7.00 (m, 3H), 6.89 (d, 1H, J = 7.5 Hz), 3.94 (s, 2H), 2.29 (s, 3H).

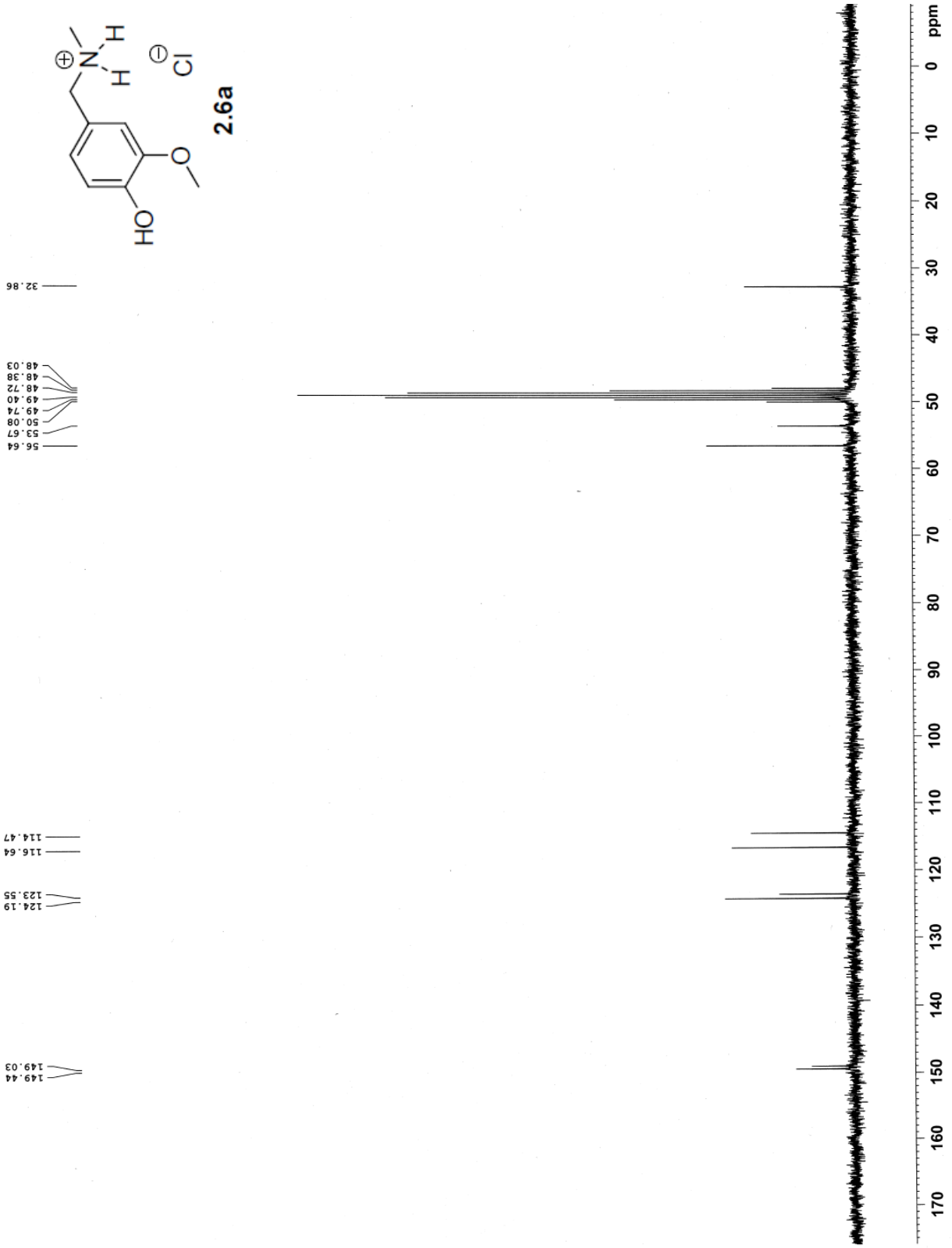
APPENDIX A

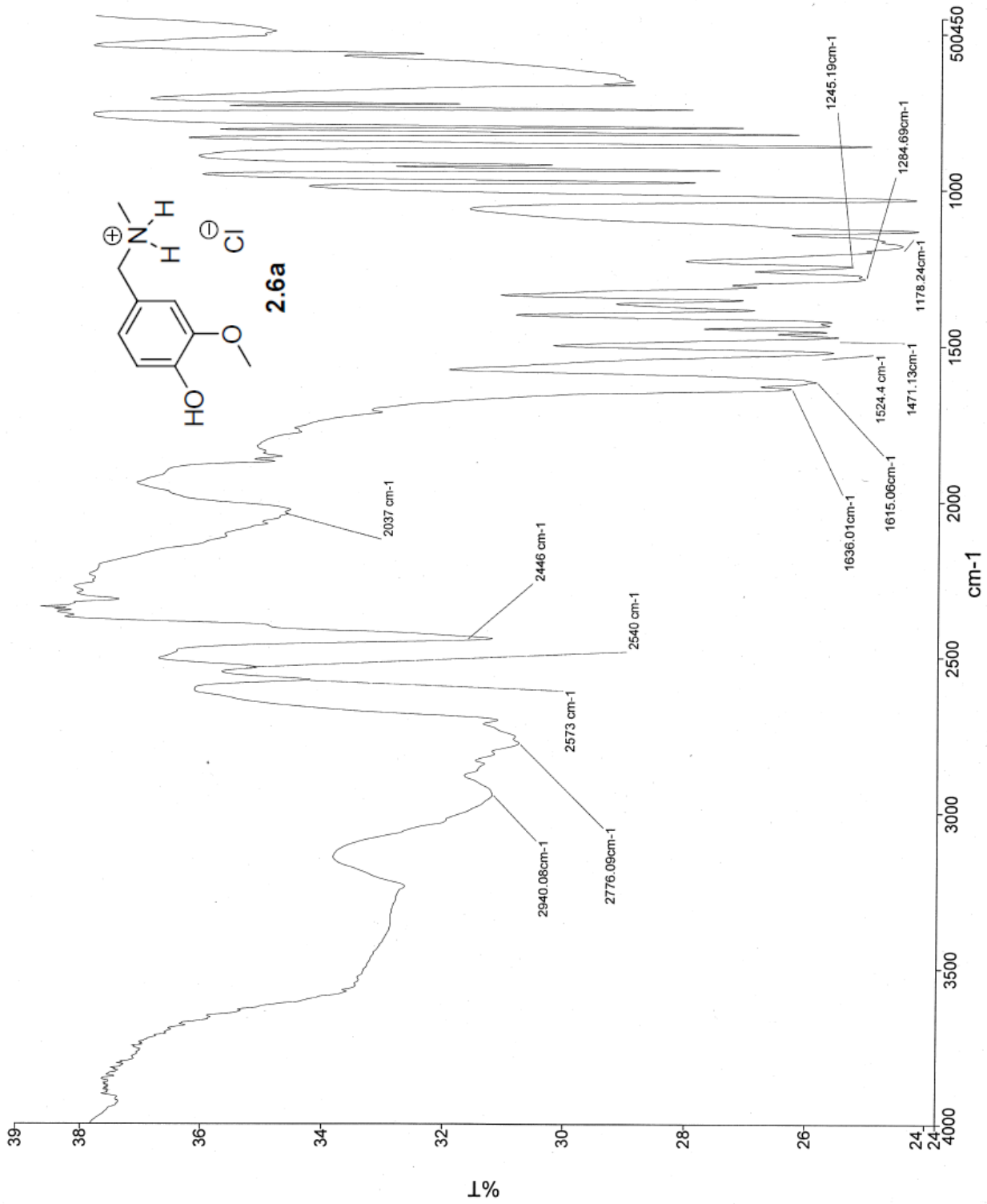
^{1}H AND ^{13}C NMR SPECTRA

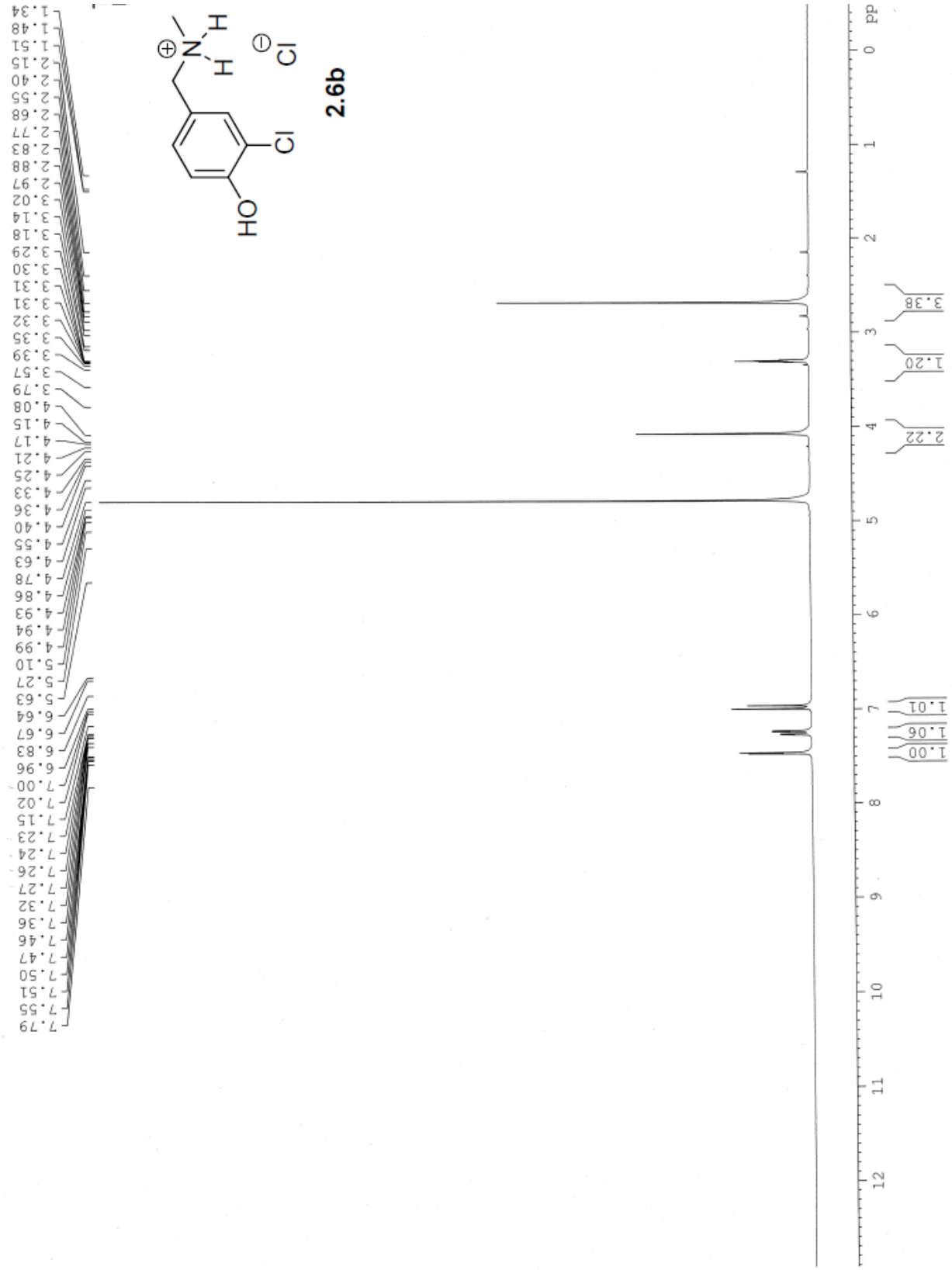
AND

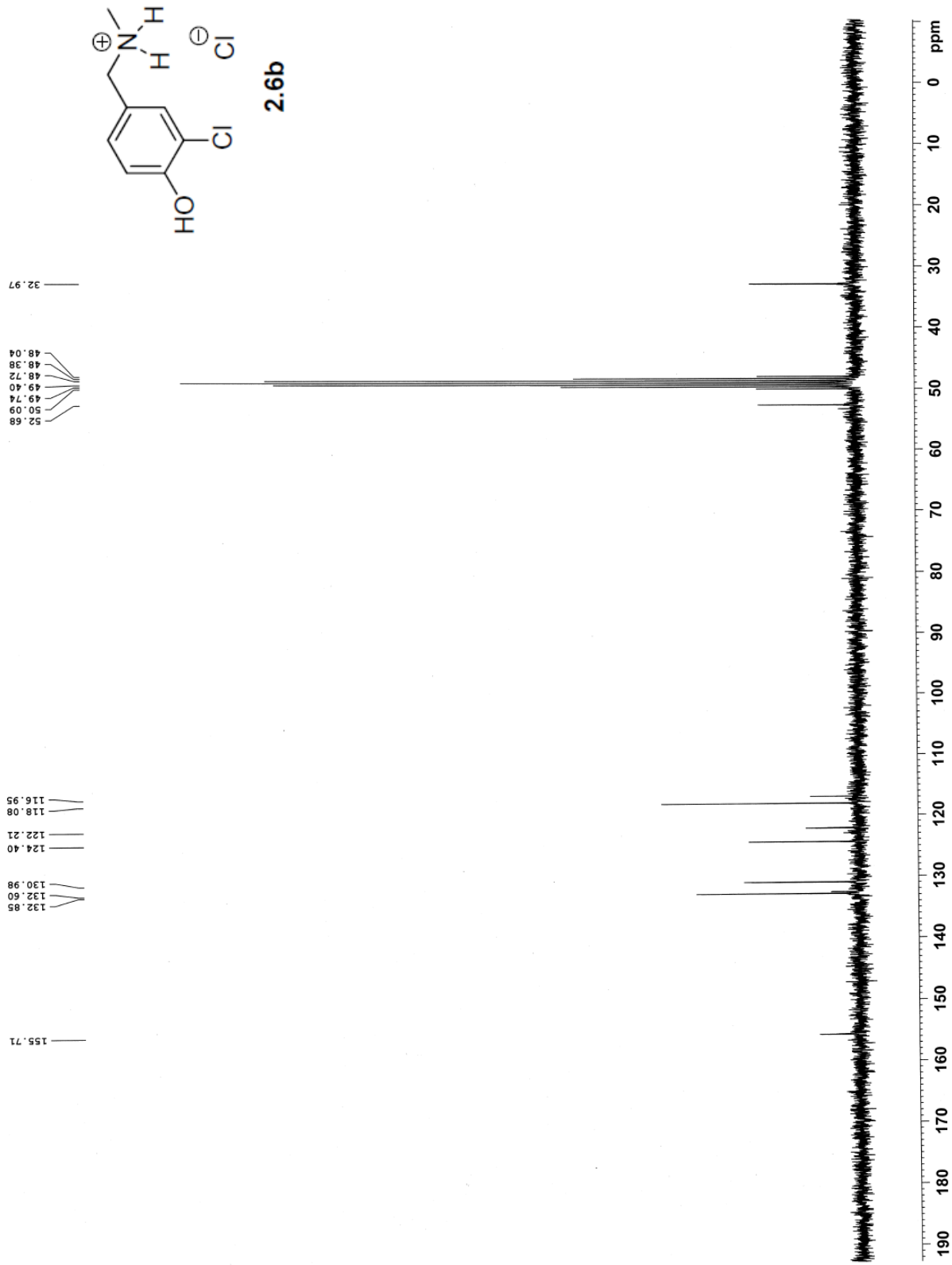
IR SPECTRA

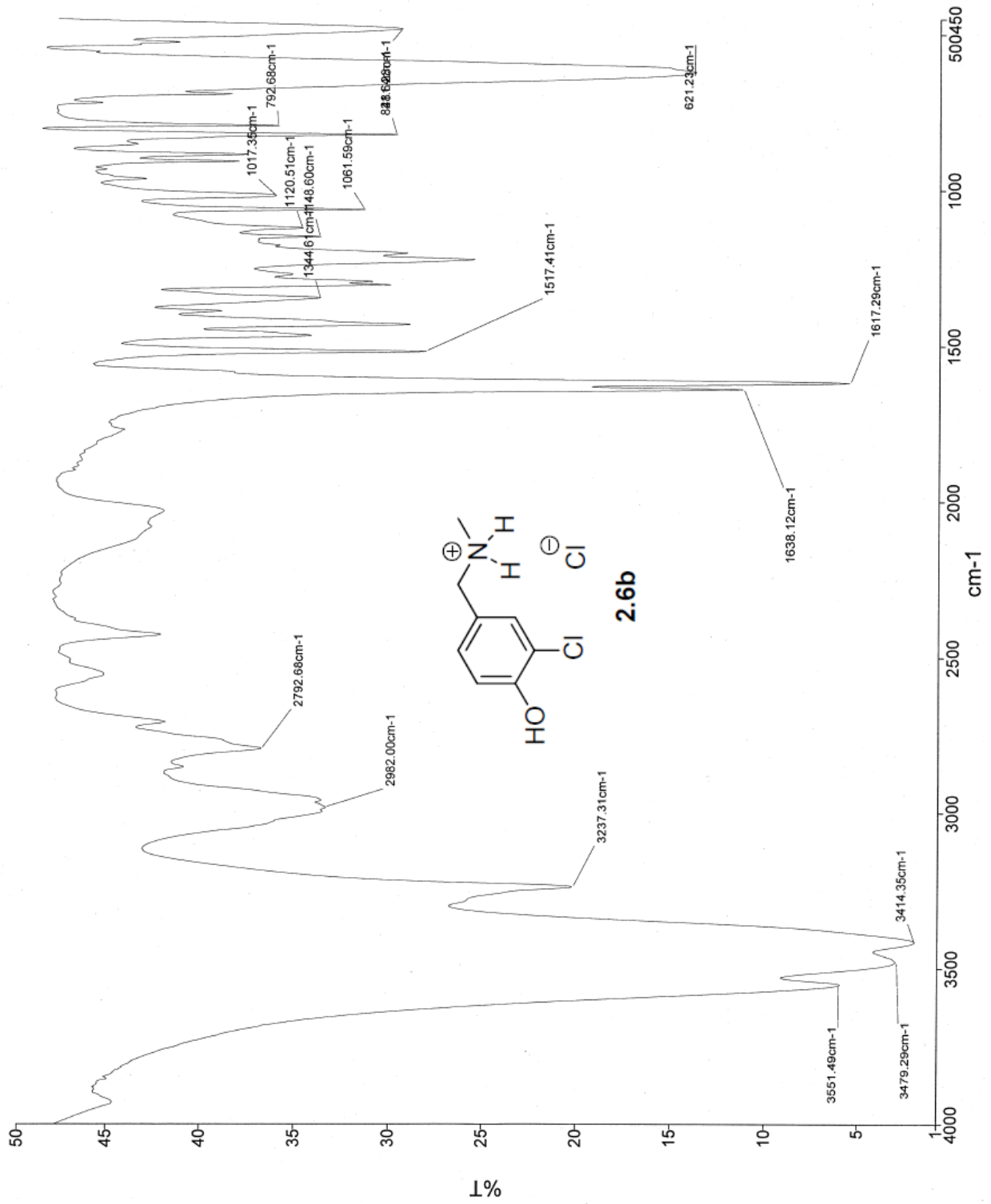

2.6a
HO
O
Cl
6.95
6.94
6.81
6.80
6.78
6.77
6.73
6.70
4.78
3.95
3.77
3.19
3.18
3.18
3.19
3.17
1.00
2.06
3.08
1.25
3.20
2.07
ppm

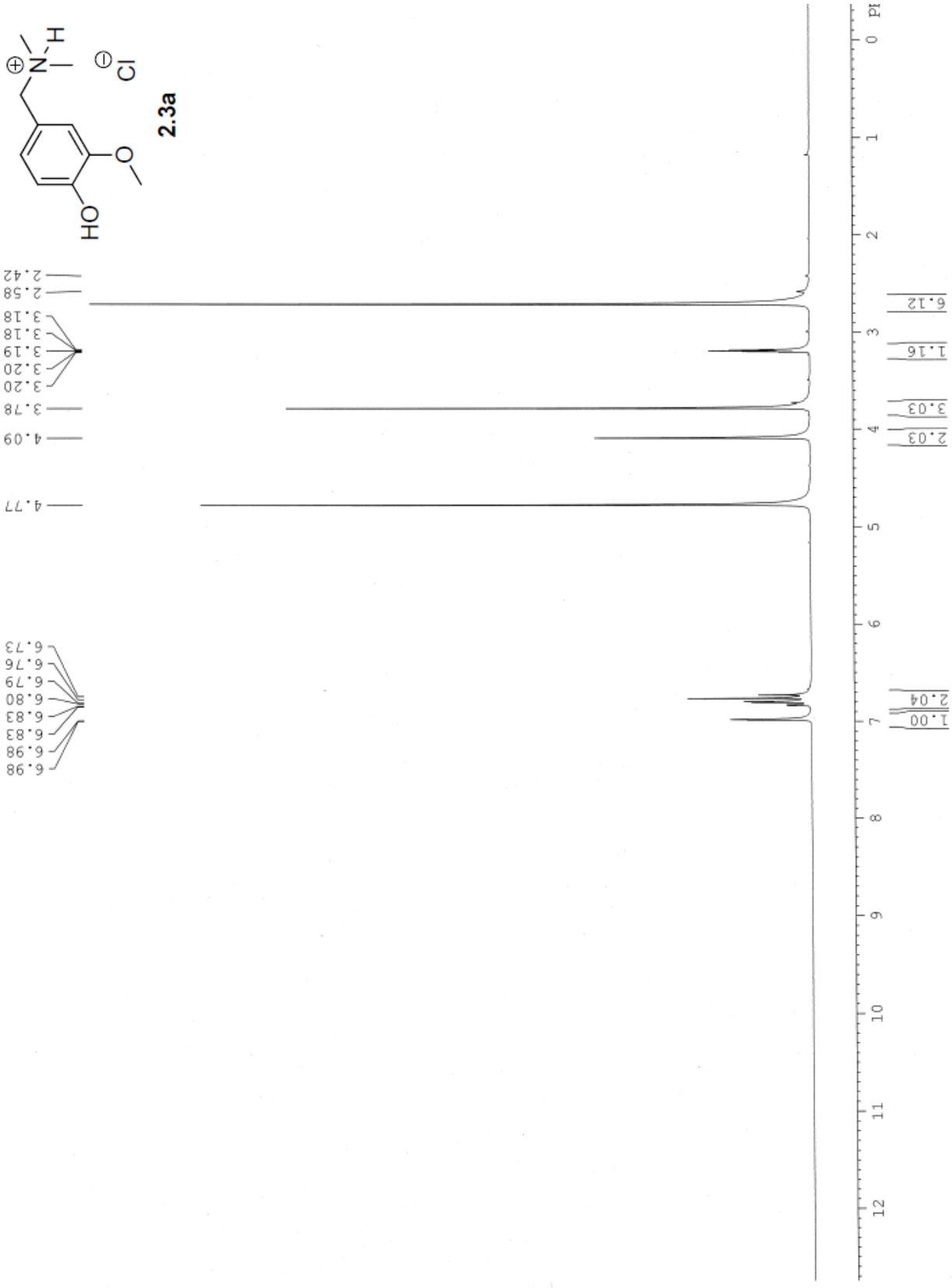

2.6a
32.86
48.03
48.38
48.72
49.40
49.74
50.08
53.67
56.64
114.47
116.64
123.55
124.19
149.03
149.44
ppm

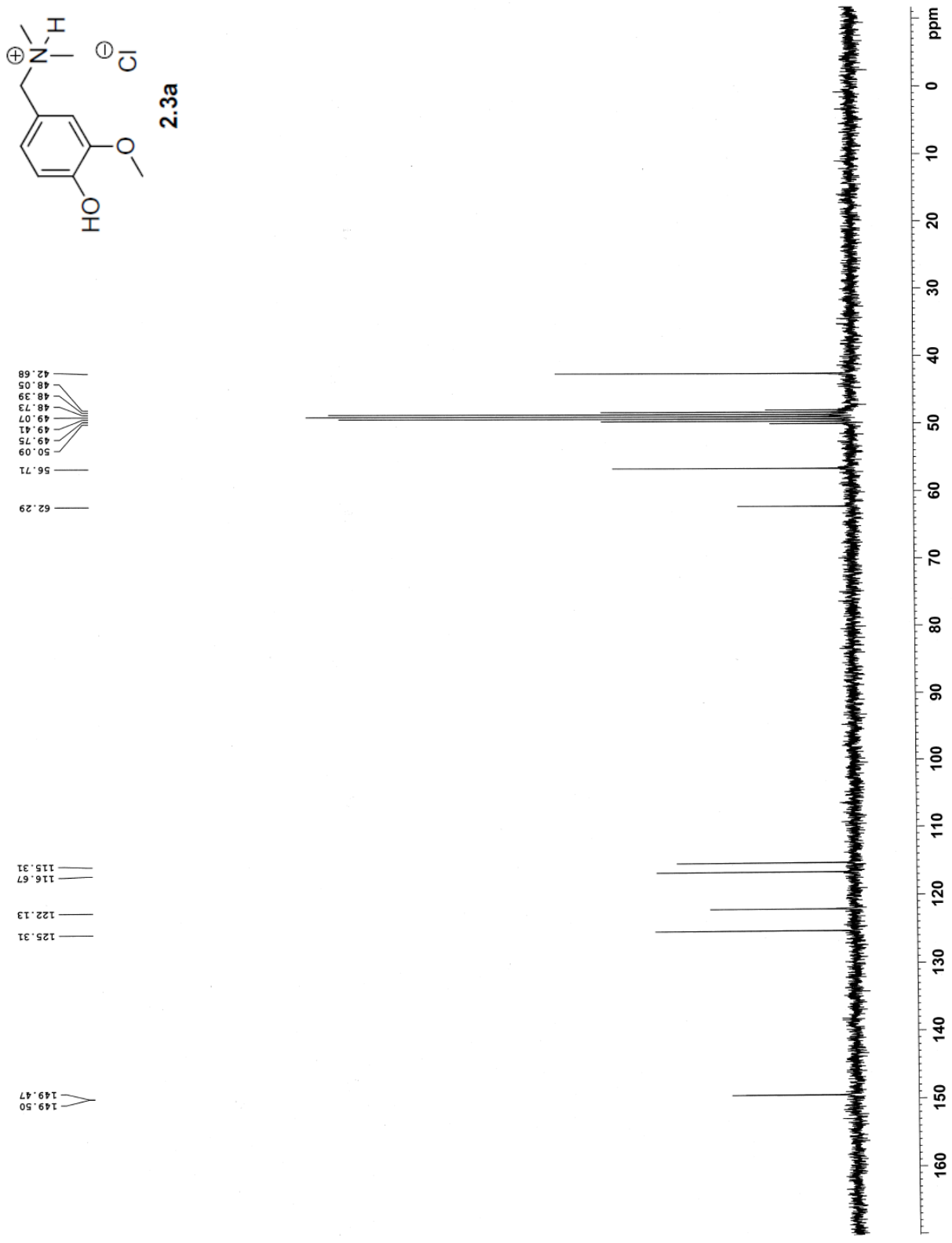

2.6a
HO
O
Cl
2037 cm-1
2446 cm-1
2540 cm-1
2573 cm-1
2940.08cm-1
2776.09cm-1
1636.01cm-1
1615.06cm-1
1524.4 cm-1
1471.13cm-1
1245.19cm-1
1284.69cm-1
1178.24cm-1
%T
cm-1

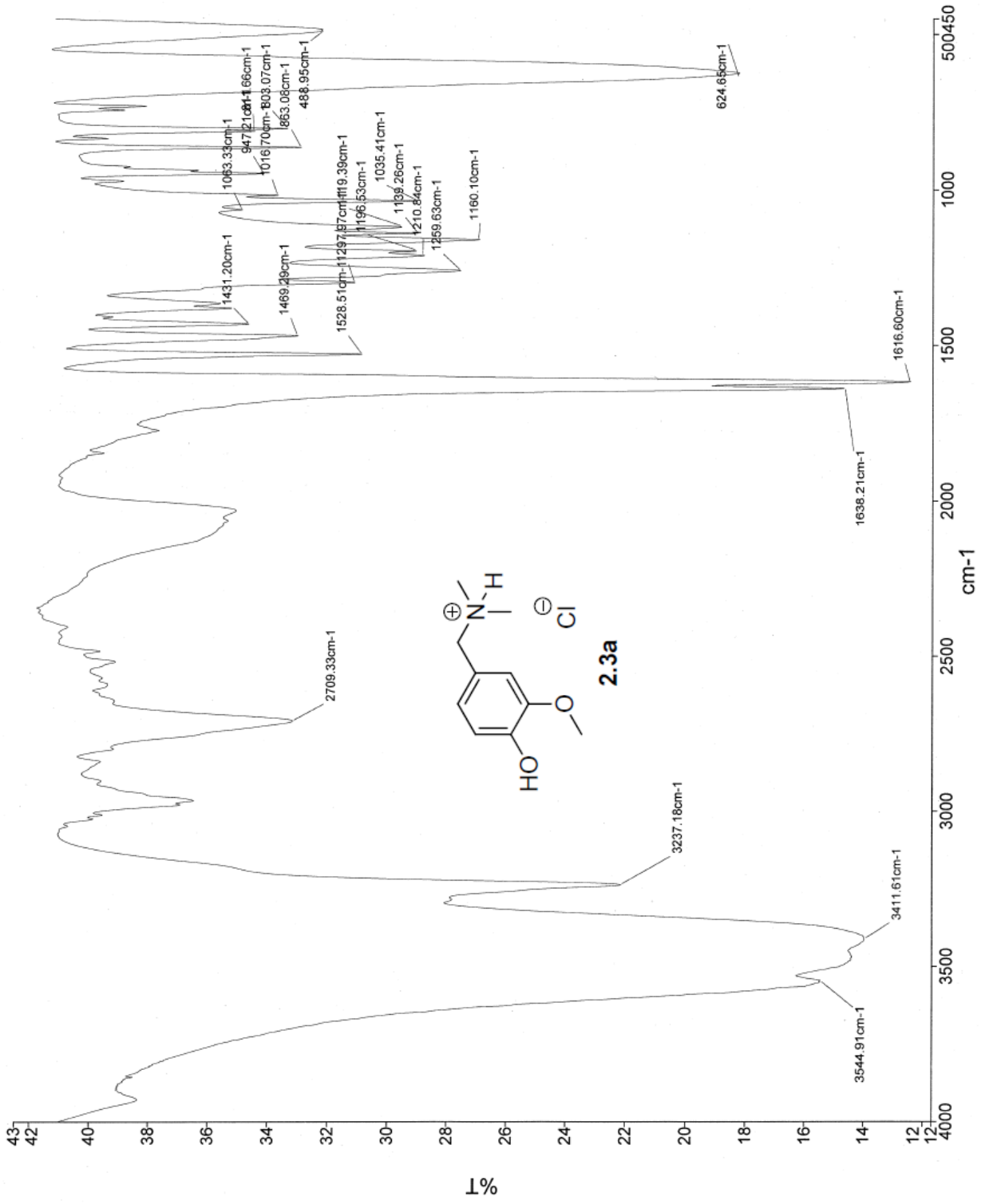

2.6b
Cl
HO
Cl
H
H
N
1.34
1.48
1.51
2.15
2.40
2.55
2.68
2.77
2.83
2.88
2.97
3.02
3.14
3.18
3.29
3.30
3.31
3.31
3.32
3.35
3.39
3.57
3.79
4.08
4.15
4.17
4.21
4.25
4.33
4.36
4.40
4.55
4.63
4.78
4.86
4.93
4.94
4.99
5.10
5.27
5.63
6.64
6.67
6.83
6.96
7.00
7.02
7.15
7.23
7.24
7.26
7.27
7.32
7.36
7.46
7.47
7.50
7.51
7.55
7.79
3.38
1.20
2.22
1.01
1.06
1.00
ppm

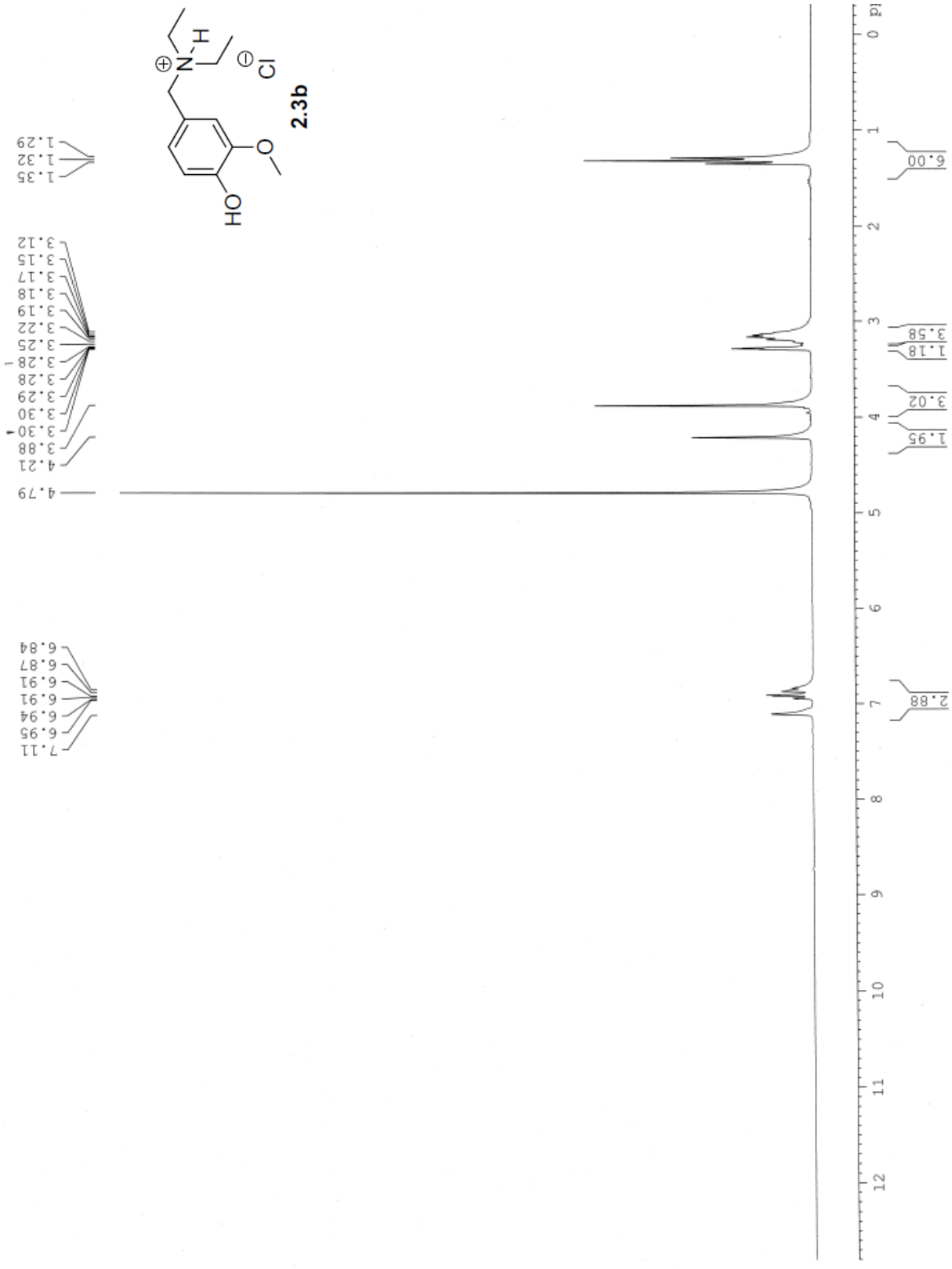

2.6b
Cl
Cl
HO
32.97
48.04
48.38
48.72
49.40
49.74
50.09
52.68
116.95
118.08
122.21
124.40
130.98
132.60
132.85
155.71
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190

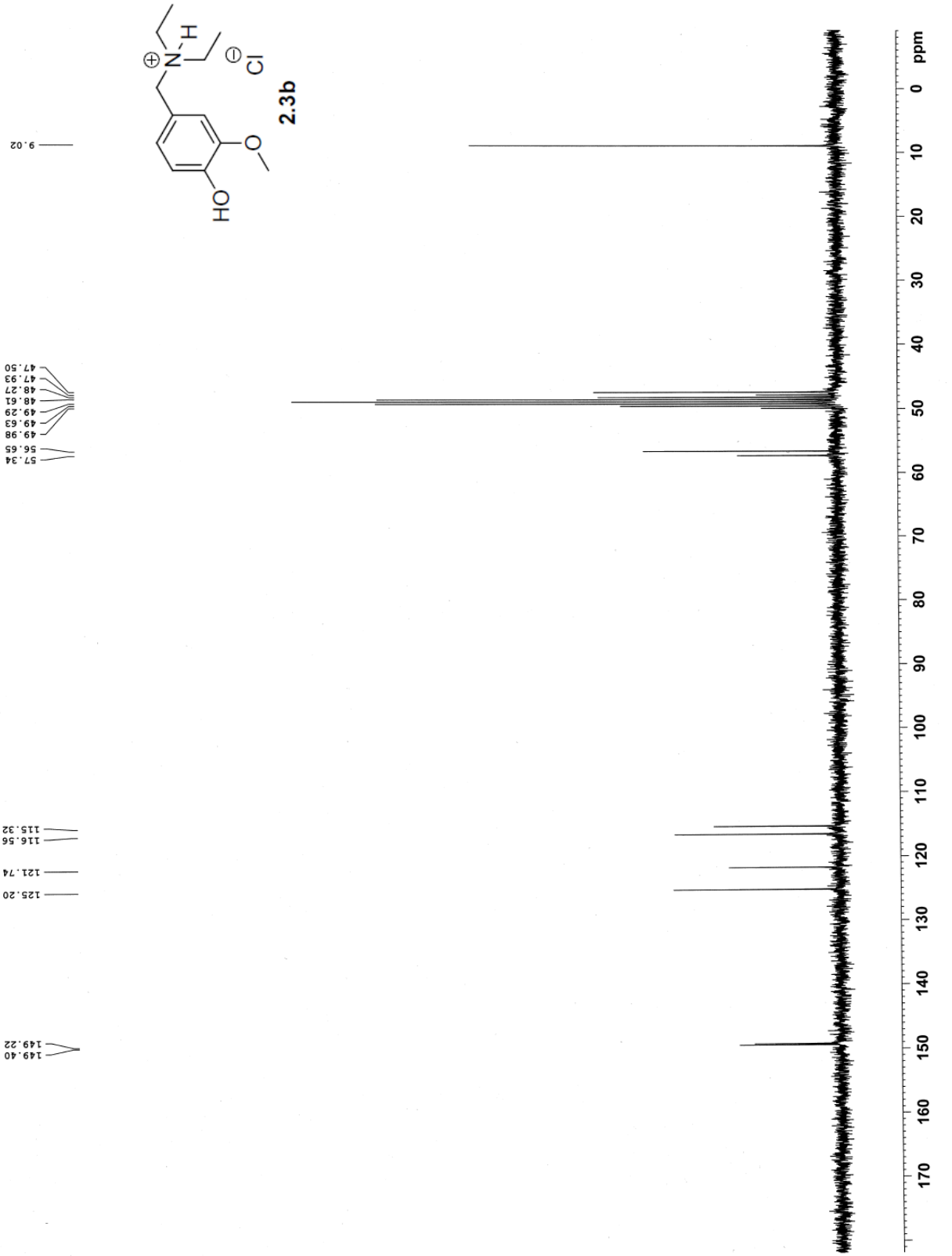

%T
cm-1
3551.49cm-1
3479.29cm-1
3414.35cm-1
3237.31cm-1
2982.00cm-1
2792.68cm-1
1638.12cm-1
1617.29cm-1
1517.41cm-1
1344.61cm-1
1148.60cm-1
1120.51cm-1
1061.59cm-1
1017.35cm-1
792.68cm-1
621.23cm-1
2.6b

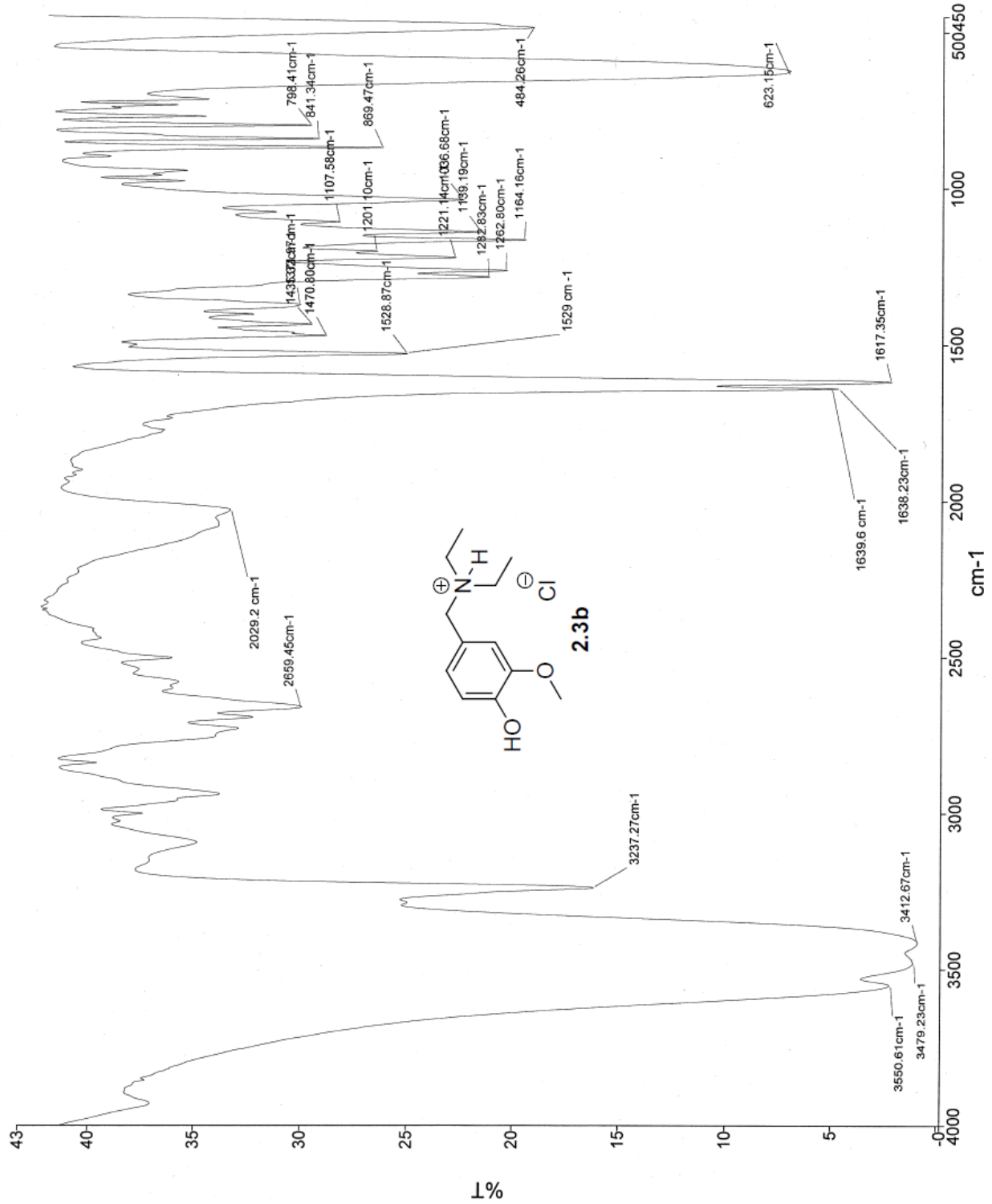

2.3a
2.42
2.58
3.18
3.18
3.19
3.20
3.20
3.78
4.09
4.77
6.73
6.76
6.79
6.80
6.83
6.83
6.98
6.98
6.12
1.16
3.03
2.03
1.00
2.04


2.3a
42.68
48.05
48.39
48.73
49.07
49.41
49.75
50.09
56.71
62.29
115.31
116.67
122.13
125.31
149.50
149.47
ppm

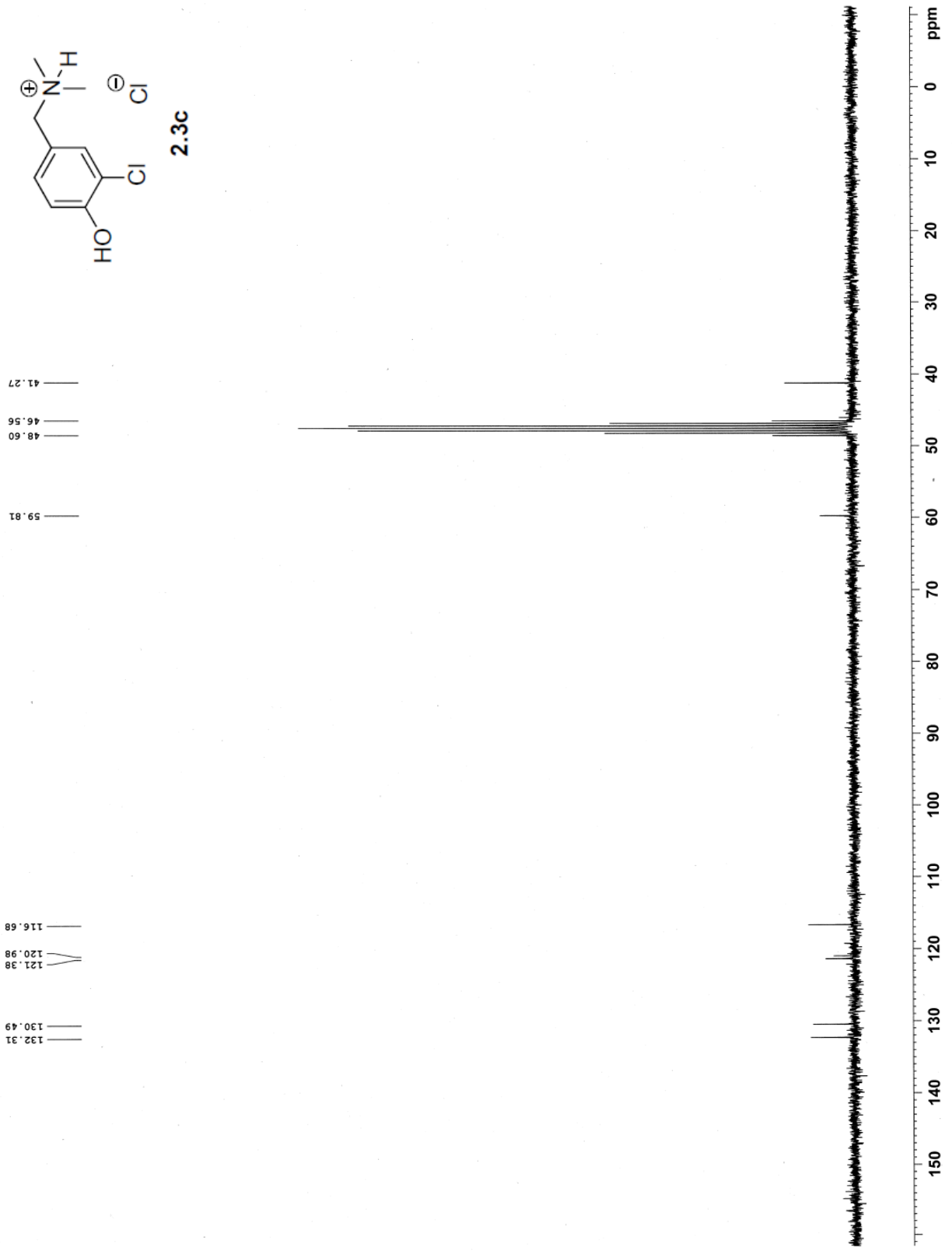

%T
cm-1
3544.91cm-1
3411.61cm-1
3237.18cm-1
2709.33cm-1
1638.21cm-1
1616.60cm-1
1528.51cm-1
1469.29cm-1
1431.20cm-1
1160.10cm-1
1259.63cm-1
1210.84cm-1
1139.26cm-1
1035.41cm-1
1196.53cm-1
1119.39cm-1
1083.33cm-1
1016.70cm-1
947.21cm-1
863.08cm-1
803.07cm-1
624.65cm-1
488.95cm-1
2.3a

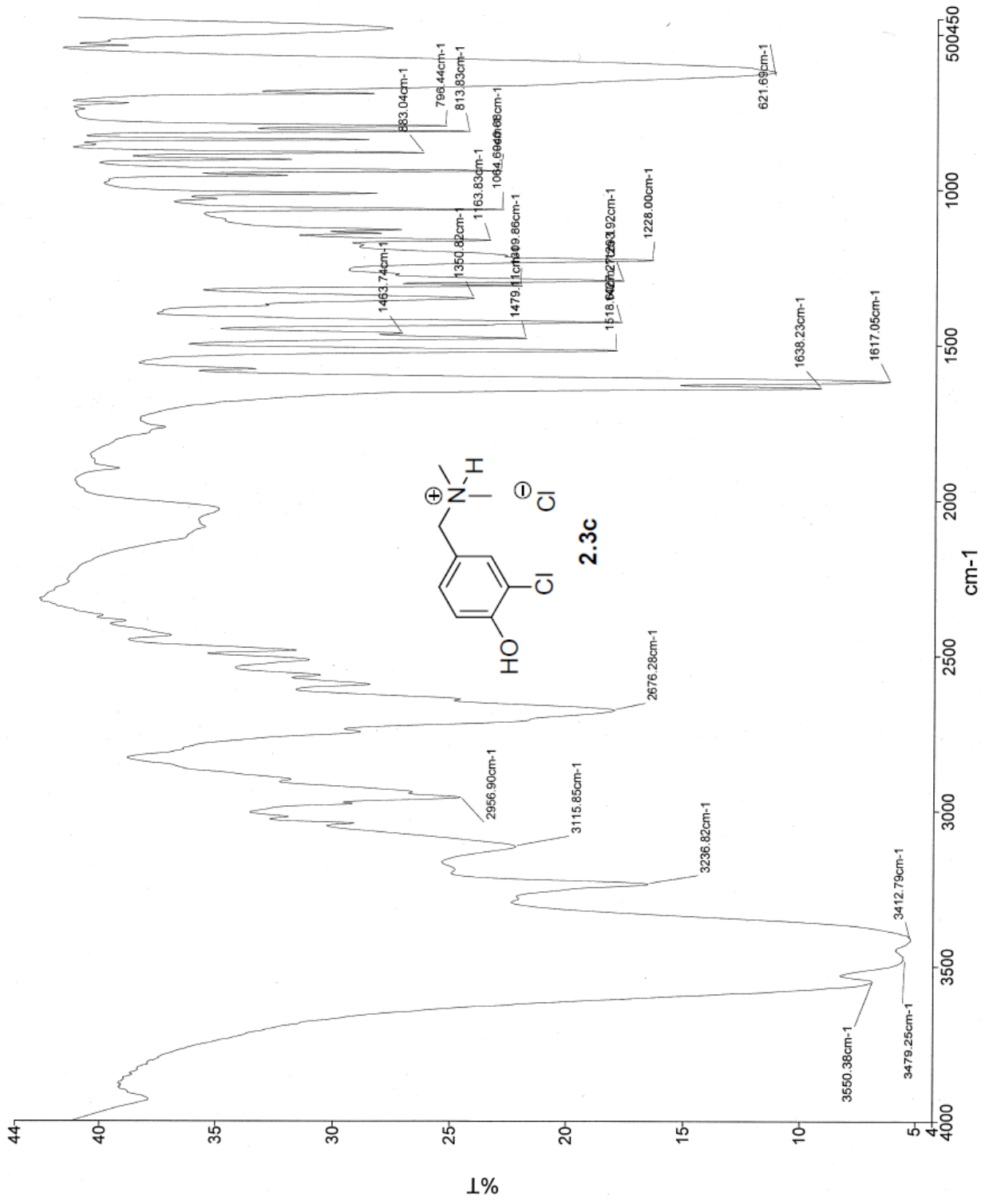

2.3b
HO
O
H
N
Cl
1.29
1.32
1.35
3.12
3.15
3.17
3.18
3.19
3.22
3.25
3.28
3.28
3.29
3.30
3.30
3.88
4.21
4.79
6.84
6.87
6.91
6.91
6.94
6.95
7.11
6.00
1.18
3.58
3.02
1.95
2.88

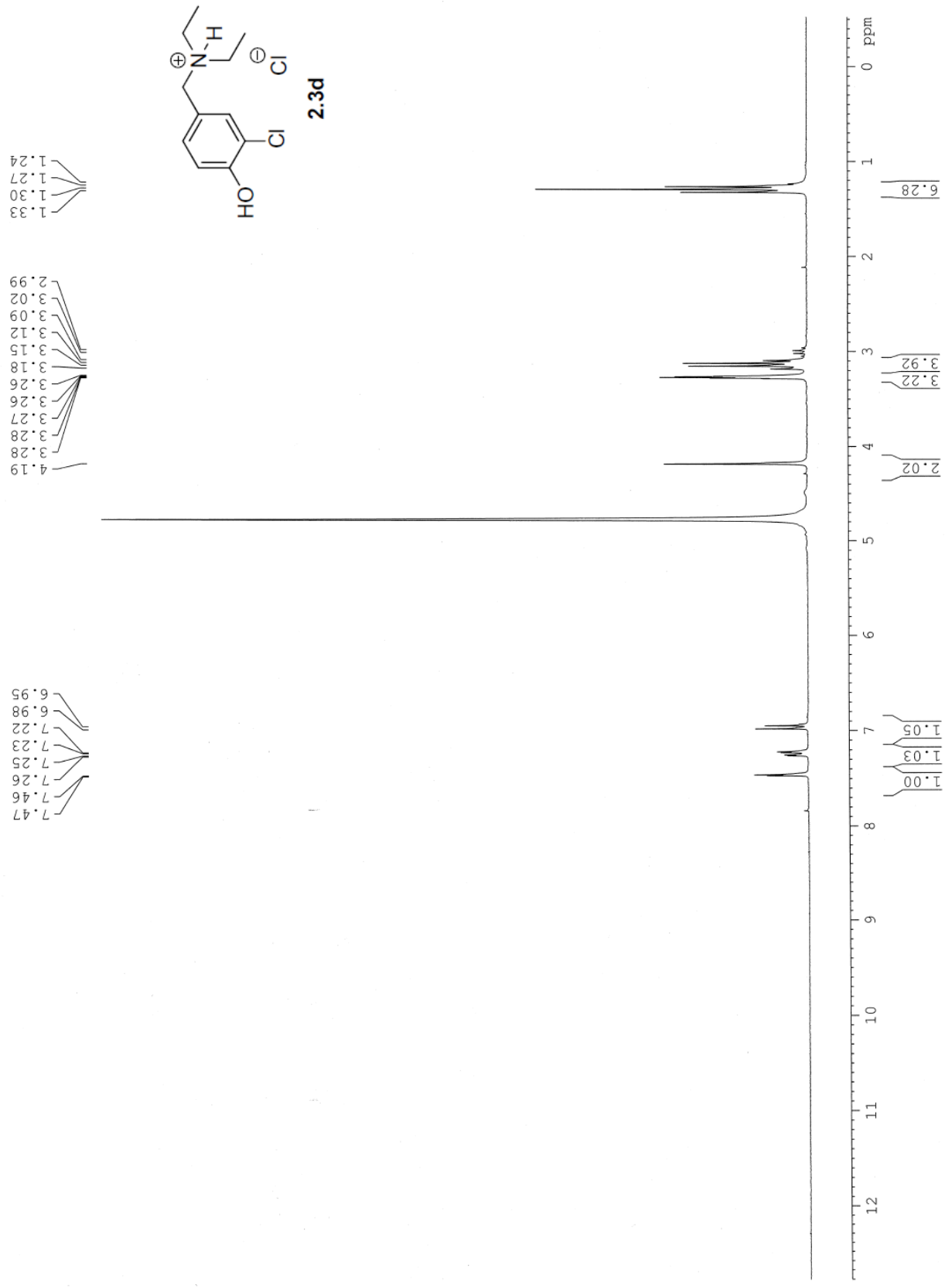

2.3b
HO
O
Cl
9.02
47.50
47.93
48.27
48.61
49.29
49.63
49.98
56.65
57.34
115.32
116.56
121.74
125.20
149.22
149.40
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170

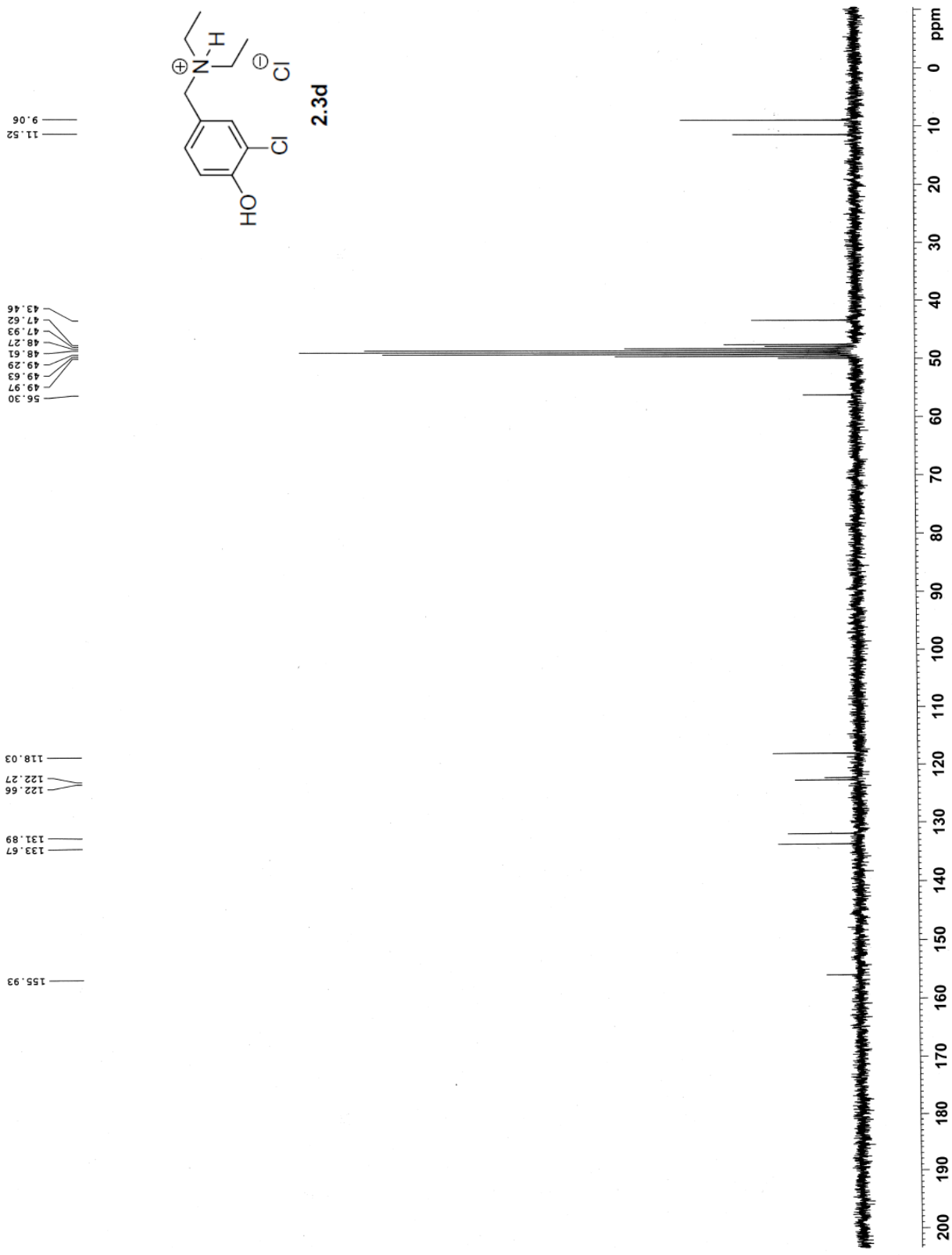

%T
cm-1
3550.61cm-1
3479.23cm-1
3412.67cm-1
3237.27cm-1
2659.45cm-1
2029.2 cm-1
1639.6 cm-1
1638.23cm-1
1617.35cm-1
1529 cm -1
1528.87cm-1
1470.80cm-1
1262.80cm-1
1164.16cm-1
1107.58cm-1
869.47cm-1
841.34cm-1
798.41cm-1
623.15cm-1
484.26cm-1
HO
Cl
2.3b

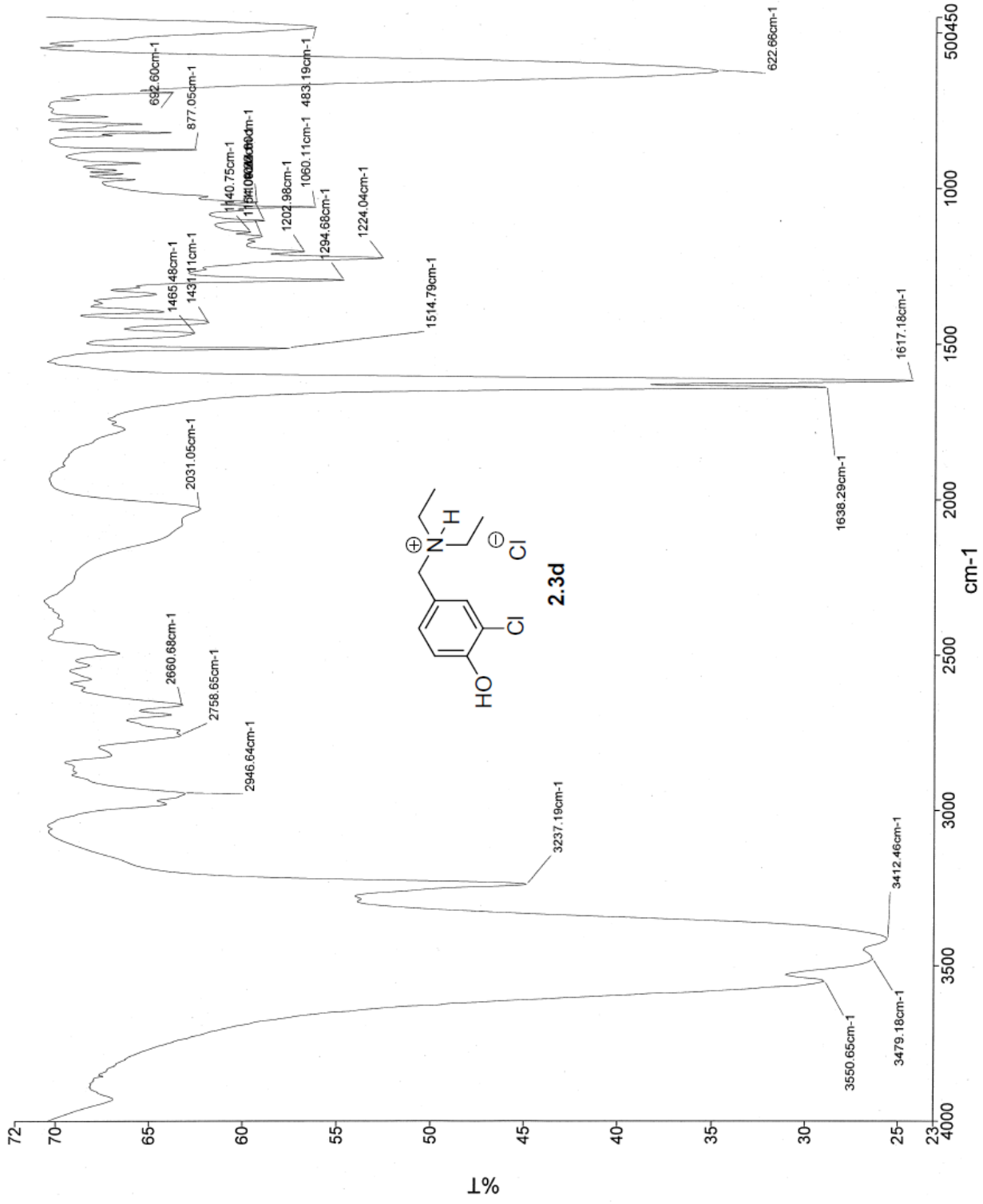
2.3c
2.67
2.79
3.26
3.27
3.27
3.28
4.18
4.78
6.95
6.99
7.24
7.24
7.27
7.28
7.47
7.48
0.94
1.00
0.97
1.99
0.60
6.00


2.3c
41.27
46.56
48.60
59.81
116.68
120.98
121.38
130.49
132.31
ppm


%T
cm-1
2.3c
3550.38cm-1
3479.25cm-1
3412.79cm-1
3236.82cm-1
3115.85cm-1
2956.90cm-1
2676.28cm-1
1638.23cm-1
1617.05cm-1
1518.64cm-1
1479.11cm-1
1463.74cm-1
1350.82cm-1
1228.00cm-1
1163.83cm-1
1064.68cm-1
883.04cm-1
813.83cm-1
796.44cm-1
621.69cm-1


2.3d
Cl
HO
1.33
1.30
1.27
1.24
2.99
3.02
3.09
3.12
3.15
3.18
3.26
3.26
3.27
3.28
3.28
4.19
6.95
6.98
7.22
7.23
7.25
7.26
7.46
7.47
6.28
3.92
3.22
2.02
1.05
1.03
1.00
ppm


2.3d
9.06
11.52
43.46
47.62
47.93
48.27
48.61
49.29
49.63
49.97
56.30
118.03
122.27
122.66
131.89
133.67
155.93
ppm


692.60cm-1
877.05cm-1
1440.75cm-1
1202.98cm-1
1060.11cm-1
483.19cm-1
1465.48cm-1
1431.11cm-1
1294.68cm-1
1224.04cm-1
1514.79cm-1
622.66cm-1
1617.18cm-1
2031.05cm-1
2660.68cm-1
2758.65cm-1
2946.64cm-1
1638.29cm-1
3237.19cm-1
3412.46cm-1
3550.65cm-1
3479.18cm-1
2.3d
%T
cm-1

REFERENCES

1. Peter, M.G. *Angew. Chem. Int. Ed. Engl.* **1989,** *28*, 555.

2. Ryan, W. V.; Thomas, R. R. P. T. *Tetrahedron* **2002,** *58*, 5367-5405.

3. Wan, P. *J. Am. Chem. Soc.* **1995,** 117, 5369-5370.

4. Myers, J.K.; Cohen, J.D.; Widlanski, T.S.; *J. Am. Chem. Soc.* **1995,** *117*, 11049.

5. Rajski, S. R.; Williams, R. M.; *Chem. Rev.* **1998,** *98*, 2723.

6. Nguyen, C.; Blanco, J.; Mazaleyrat, J.P.; Krust, B.; Callebaut, C.; Jacotot, E.; Hovanessian, A. G.; Wakselman, M. *J. Med. Chem.* **1998,** *41*, 2100.

7. Stowell, J.K., Widlanski, T.S., Kutateladze, T.G.; Raines, R. T. *J. Org. Chem.* **1995,** *60*, 6930.

8. Wang, H.; Wang, Y.; Han, K.; Peng, X.; *J. Org. Chem.* **2005,** *70*, 4910-4917.

9. Freccero. M.; *Mini-Rev. in Org. Chem.*, **2004,** *1*, 403-415

10. Modica, E.; Zanaletti, R.; Freccero, M.; Mella, M. *J. Org. Chem.* **2001,** *66*, 41.

11. Chiang, Y. A.; Kresge, J.; Zhu, Y. *J. Am. Chem. Soc.* **2002,** *123*, 717.

12. Bakke, B. A.; McIntosh, M. C.; Turnbull, K. D. *J. Org. Chem.* **2005,** *70*, 4338-4345.

13. Dyall, L.K.; Winstein, S. *J. Am. Chem. Soc.* **1971,** *94*, 2196-2199

14. Rabin, O.; Vigalok, A.; Wilstein, D. *J. Am. Chem. Soc.* **1998,** *120*, 7119-7120

15. Filar, L. J.; Winstein, S. *Tetrahedron Lett.* **1960,** *25*, 9-16.

16. Hudgens, T. L. Ph.D. Thesis, University of Arkansas, Fayetteville, Arkansas, 2002.

17. Veldhuyzen, W. F.; Shallop, A. J.; Jones, R. A.; Rokita, S. E. *J. Am. Chem. Soc.* **2001,** *123*, 11126-11132.

18. Weinert, E. E.; Frankenfield, K. N.; Rokita, S. E. *Chem. Res. Toxicol.* **2005**, *18*, 1365-1370.

19. Weinery, E. E.; Dondi, R.; Dolloredo-Melz, S.; Frankenfield, K. N.; Mitchell, C. H.; Freccero, M.; Rokita, S. E. *J. Am. Chem. Soc.* **2006,** *128*. 11940-11947

20. Zhou, Q.; Turnbull. *J. Org. Chem.***2001,** *66*, 7072-7077